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x 16
x 6
=1 kilo

a too common denominator

16 small oranges weigh 1 kilo.
6 large oranges weigh 1 kilo.

Both lots of oranges seem the same when measured by an arbitrary standard —*weight*. Actually, however, the small oranges may contain more juice or a greater concentration of Vitamin C.

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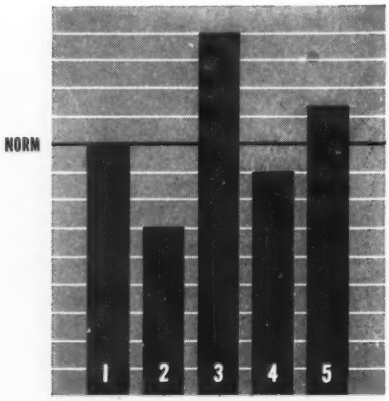

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SPONTANEOUS AND EXPERIMENTALLY INDUCED UPTAKE OF RADIOACTIVE IODINE IN METASTASES FROM THYROID CARCINOMA: A PRELIMINARY REPORT*†

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METASTATIC carcinoma of the thyroid can be successfully treated with radioiodine when the lesions concentrate this isotope (1). The usefulness of this method depends upon the percentage of cases in which such concentration can be demonstrated. A technique of inducing uptake of radioiodine in metastases that previously showed none would increase the usefulness of the method. Clinically we have been successful in inducing such uptake in several cases with subsequent amelioration of the disease.

In a recent publication by Marinelli *et al.* (2), it is reported that "approximately 15 per cent of thyroid cancers may be expected to accumulate radioactive iodine in some degree." This conclusion is based on findings in a selected group of 19 cases modified by an estimation of relative frequency of various types of thyroid carcinoma.

The present paper is a report on 14 unselected cases of metastatic thyroid carcinoma studied at Montefiore Hospital up to June 1947.

Received for publication March 18, 1948.

* Presented at the Twenty-ninth Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, New Jersey, June 7, 1947.

† This work was aided by grants from the Dazian Foundation for Medical Research and from Mr. and Mrs. M. Lincoln Schuster.

METHOD

Tracer doses ranging from 500 to 2000 microcuries of radioactive iodine (I^{131}) were administered orally to patients on nonrestricted diets. All urines were collected for at least the first forty-eight hours and studied for radioactive iodine content. Forty-eight hours after the dose was administered,

RADIOIODINE UPTAKE BY METASTASES FROM THYROID CARCINOMA

PATIENTS	GEIGER COUNTER ON ADMISSION	RADIOAUTOGRAPHS
R.A.	-	
W.B.	-	
A.B.	-	
B.B.	+	+
S.C.	-	
M.F.	-	
J.F.	-	
L.H.	+	+
L.L.	-	+
L.O.	+	+
M.S.	+	+
A.V.	-	
C.W.	-	
D.Y.	+	+
Totals: 14	5 + 9 - 36% 64%	6 + 8 not done. 43% 57%

FIG. 1

in vivo readings were taken with a Geiger-Mueller counter to determine the degree of localization of radioactive iodine in the thyroid gland and the metastases. The cases listed as positive for uptake in metastases as indicated by external Geiger counter measurements are those in which there was no doubt that the lesion contained a significantly higher concentration of radioiodine than a control area. The cases listed as negative are those in which it was not possible to demonstrate such a concentration.

In the later cases of the series, quantitative measurements were made to determine the percentage of the administered dose concentrated in the gland.

In cases submitted to biopsies radioautographic studies were carried

CASES ORIGINALLY "NEGATIVE" TREATED WITH T.S.H. OR I^{*}

PATIENTS	TREATMENT	STATUS AFTER Rx
R.A.	I^{*} (103 mc. I^{131} - 80% excreted in first 4 days)	Unknown, patient left hospital.
W.B.	T.S.H. (1 cc. Armours T.S.H. daily 4 - 5 weeks)	Negative
A.B.	T.S.H. (2 cc. Armours T.S.H. daily 17 times)	+
J.F.	I^{*} (100 mc. I^{131} - 82% excreted in first 4 days)	+
L.L.	I^{*} (10 mc. I^{131} ~ 70% excreted in first 4 days)	+

The other four negative cases were not treated in any way.

FIG. 2

out. A "large tracer" or a therapeutic dose of radioiodine was administered, usually forty-eight hours before the procedure. The tissue obtained at biopsy was imbedded in paraffin and sections cut, ten microns or less in thickness. The paraffin on at least 3 sections from each block was removed with xylol, an estimate of activity was obtained in a Geiger counter and the slides were exposed to a photographic emulsion for a variable period of time as determined from counter measurements. After the necessary exposure time, the slides were removed from the emulsion and stained and the films developed according to usual photographic technique.

RESULTS

As indicated in Figure 1, five out of the 14 cases showed uptake in at least one metastatic lesion the first time radioiodine was administered. Subsequently, radioautographs were made from tissue removed from six of the

14 patients. In all these cases the radioautograph showed definite concentration of radioactive iodine in the metastatic tissue. In one, external measurements had not indicated uptake, showing that the radioautographic technique, when feasible, is more sensitive than external measurements.

All the "positive" cases had previously had a complete or nearly complete thyroidectomy (B.B. and L.H. (3)) or a subtotal thyroidectomy followed by x-ray treatment. However, not all patients who had had partial thyroidectomy and x-ray treatment showed radioiodine uptake by metastases.

In some "negative" cases an attempt was made to induce uptake of radioiodine by pretreatment with thyrotropic hormone of the pituitary (T.S.H.) or by performing a "radiation thyroidectomy" through administration of a large dose of radioactive iodine. The results are shown in Figure 2.[1] Of the two cases treated with T.S.H. the results were positive in one and negative in the other.

Radiation thyroidectomies were attempted in 3 patients. One of these left the hospital too early for the results to be evaluated. In the other two, iodine uptake was induced in metastatic lesions. In one, J. F., there was a metastatic lesion in the skull which showed no uptake of I* at first. Neither a short series of injections of thyrotropic hormone nor a short course of thiouracil therapy caused any detectable I* uptake in this metastasis. Therefore, a 100 millicurie dose of I^{131} was administered. Six weeks later a tracer dose was given and Geiger counter readings showed definite uptake in the skull lesion. The other patient, L. L., originally showed no uptake in a lesion in the sternum. A 10 millicurie dose of I* was given. The next tracer dose about 2 months later showed uptake in this lesion.

Figures 3, 4, 5 and 6 are microphotographs of slides from the tumor tissue of patients listed in Figure 2 before any treatment was instituted. The last three are examples of highly differentiated thyroid tumors which did not concentrate radioiodine until stimulated by thyrotropic hormone, directly by injections of T.S.H. or indirectly by thyroidectomy.

In addition, in a "positive" case, M. S., the uptake of radioiodine in the tumor tissue was increased several-fold by a course of injections of T.S.H.

On each of 8 patients who had received radioiodine therapy, 1 to 5 curves of radioiodine concentration in the blood were taken. All of them were similar in shape to the one shown in Figure 7. However, after the first day, the radioiodine concentration in the one who had a "total" thyroid-

[1] The symbol I* used in this figure and throughout the article is the standard abbreviation for radioactive iodine.

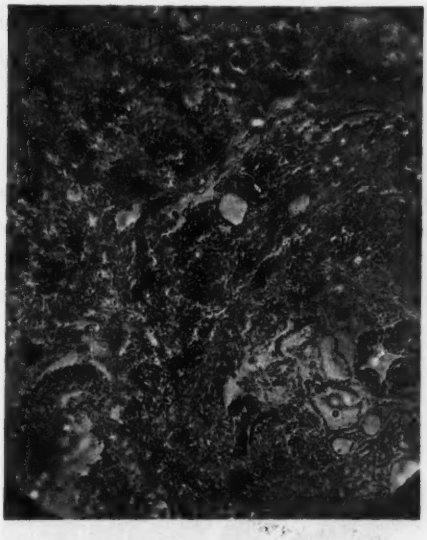

FIG. 3. Patient W. B. Section of tumor shows highly anaplastic tissue with some areas of differentiation.

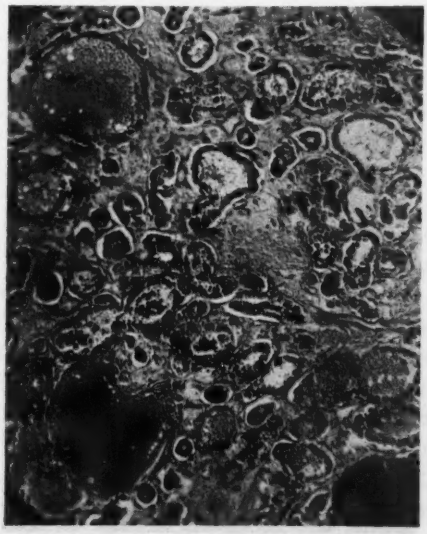

FIG. 4. Patient A. B. Section of tumor shows marked differentiation: definite follicle formation with colloid.

FIG. 5. Patient J. F. Section of tumor shows marked differentiation: follicle formation with colloid.

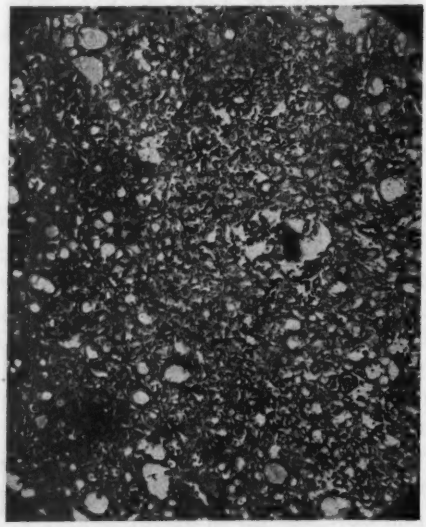

FIG. 6. Patient L. L. Section of tumor shows anaplastic areas as well as areas of varying degrees of differentiation.

ectomy was at a higher level than in any of the others. It appears that the level at which the curve flattens out may be an indication of the amount of normal thyroid tissue present in a given patient.

COMMENTS

In 1940, Seidlin (4) demonstrated the *inactivation* of *T.S.H.* by normal thyroid tissue, both in vivo and in vitro. About two years later the in vitro experiments were confirmed by Aub and his associates (5). Whatever

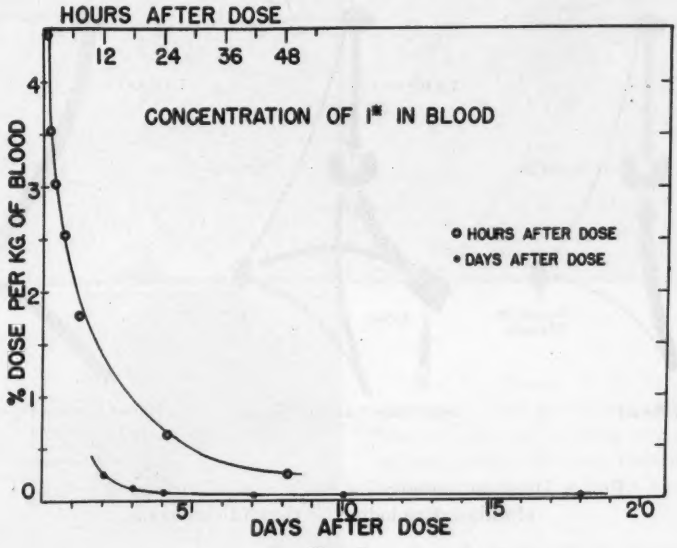

FIG. 7

the chemical reaction that takes place at the site of inactivation, the end result is stimulation of the thyroid tissue. This has been repeatedly demonstrated for *normal* thyroid tissue, both in vivo and in vitro.

Leiter, Seidlin, Marinelli and Baumann (3) have shown that at least in the two patients, B. B. and L. H., the metastases function in many respects like normal thyroid tissue. In our completely thyroidectomized patient (B. B.) intramuscular injections of thyrotropic factor were not followed by the appearance of T.S.H. in his urine. We felt justified in concluding that his metastatic thyroid carcinoma inactivated the T.S.H. (1).

In a patient who has both normal thyroid tissue and metastatic thyroid carcinoma, the administered I* goes primarily to the normal tissue. The normal thyroid also competes successfully for the T.S.H. in the blood, and consequently for the circulating or administered iodine. The above

two processes are separate reactions although the second accelerates the first. Thyroidectomy (surgical or radiation) enables the tumors to react with more T.S.H. In the process of inactivating the T.S.H. the tumor is likely to be stimulated to a higher level of functioning activity and to take up iodine (Fig. 8).

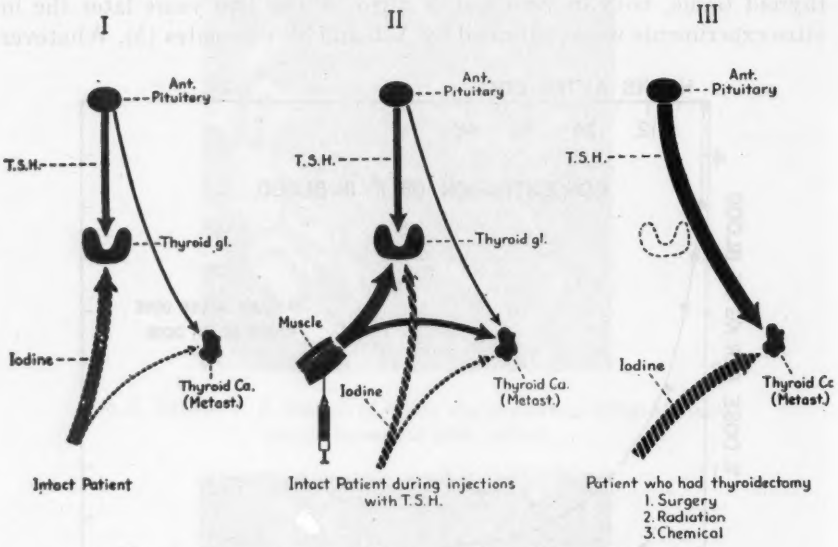

FIG. 8. Diagram representing mechanism of increasing uptake of radioactive iodine by thyroid carcinoma.

We, among others, were of the opinion that thyroid carcinoma metastases possessing an adult type of structure are more likely to pick up iodine than those with an embryonic or anaplastic structure. *This is not always true.* The slide of J. F. (Fig. 5) illustrates a tumor with well differentiated follicles containing colloid, which showed no iodine uptake until after radiation thyroidectomy. However, in patient D. Y. we find radioiodine uptake in nondifferentiated, anaplastic areas as well as in differentiated ones. This is illustrated in the slides shown in Figures 9a, 9b, 9c, prepared by Dr. Titus Evans (6) of Columbia University, using his technique of a combined histologic section and radioautograph.

The determination of the other factors which must influence the pickup of iodine by thyroid carcinoma metastases, in addition to the already demonstrated factors of histologic structure and available supply of T.S.H., requires considerable future investigation.

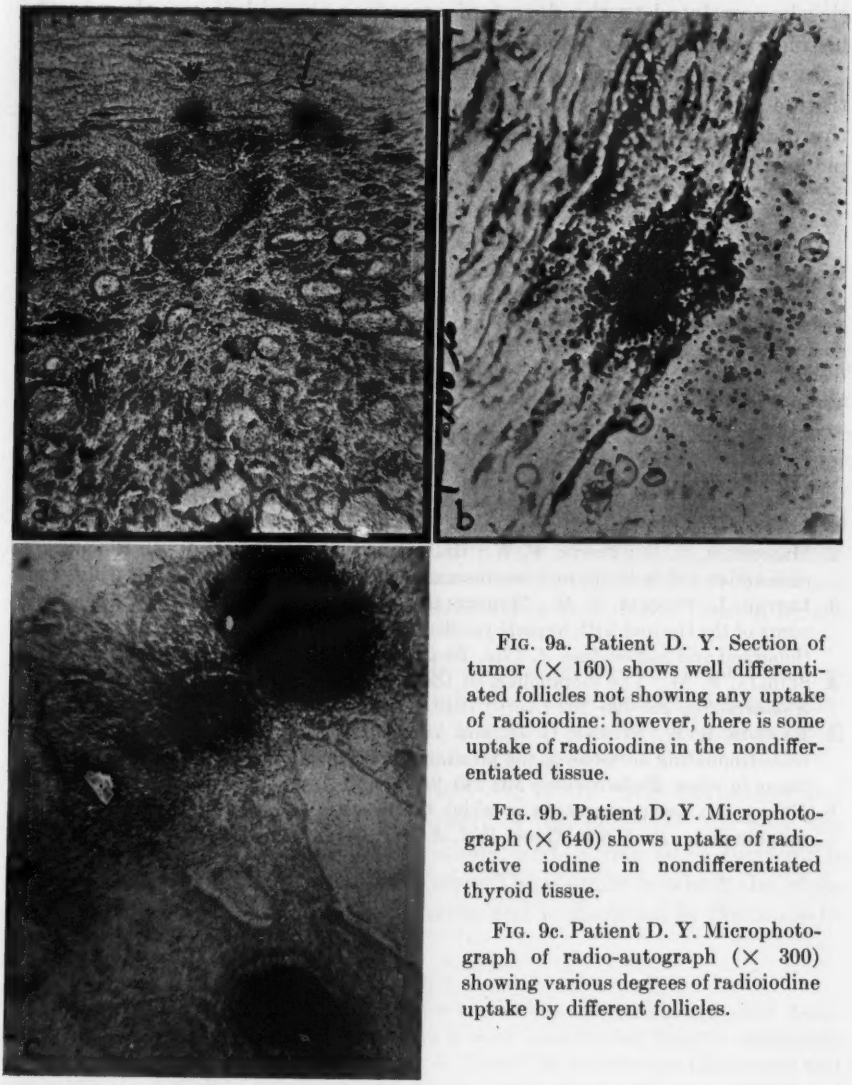

FIG. 9a. Patient D. Y. Section of tumor (× 160) shows well differentiated follicles not showing any uptake of radioiodine: however, there is some uptake of radioiodine in the nondifferentiated tissue.

FIG. 9b. Patient D. Y. Microphotograph (× 640) shows uptake of radioactive iodine in nondifferentiated thyroid tissue.

FIG. 9c. Patient D. Y. Microphotograph of radio-autograph (× 300) showing various degrees of radioiodine uptake by different follicles.

SUMMARY

In 14 unselected cases of metastatic thyroid carcinoma studied with radioactive iodine, uptake of the isotope was demonstrated in 8 (57 per cent). The radioiodine uptake by the metastatic carcinoma was more

closely correlated to the degree of preceding thyroidectomy than to the histologic structure or "type" of the tissue.

In cases in which initial studies with radioiodine do not show concentration in the metastases, such concentration may be induced by either thyroidectomy (surgical, radiation or chemical) or injections of thyrotropic hormone.

ACKNOWLEDGMENTS

The radioactive iodine used in this work was supplied by the Isotopes Division of the United States Atomic Energy Commission, Oak Ridge National Laboratory.

We wish to express our appreciation to Dr. Louis Leiter, Chief of the Medical Division, whose continued encouragement and advice made this work possible. We wish to acknowledge the cooperation of Dr. Daniel Laszlo, Chief of the Neoplastic Service. Technical assistance was given by Gaspar Marov and Barbara Eisner of the Medical Physics Research Laboratory.

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AGEING IN APPARENTLY NORMAL MEN. I. URINARY TITERS OF KETOSTEROIDS AND OF ALPHA-HYDROXY AND BETA-HYDROXY KETOSTEROIDS[1]

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THE relation of ageing to the structure and function of the various tissues and organs of the body is in need of objective study. As a move in this direction, an attempt is being made to study in a comprehensive fashion the endocrine status of 57 apparently normal men, with a range in age of 21 to 75 years. It is our further intention to describe the extent to which the endocrine state, as reflected in urinary titers of certain steroid hormones and gonadotropins, is correlated with the degree of development and maintenance of slightly more than a score of special items which are either secondary sex characters or influenced in some lesser fashion by sex hormones.

The present account pertains to the total amounts of ketosteroids excreted by 51 men in this series and to the respective proportions of alpha- and beta-ketosteroids. Brief mention is made, however, of the medical examination and battery of tests used in this survey.

It should be noted at the outset that one of the most important and certainly one of the most difficult aspects of this endeavor has been the selection of a group of normal individuals; and, above all, a *comparable* group of young, middle-aged and old men. The manner in which the problem has been met is of prime consequence and is discussed in the immediately succeeding paragraphs.

Received for publication December 2, 1947.

[1] The term "ketosteroid" is used in preference to the more commonly used designation, "17-ketosteroid." The use of this term is based upon the fact that the compounds recovered by the methods employed include 3-, 17- and 20-ketosteroids (Lieberman and associates (1). The alpha-ketosteroids refer to the hydroxy compounds not precipitated upon the addition of digitonin to the neutral ketonic fraction. The beta-ketosteroids include hydroxy compounds that are precipitated upon treatment with digitonin.

[2] These data were presented before the American Association of Anatomists in 1947 (2).

[3] This work has been supported in part by grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and from the Nutritional Products Foundation, Inc

MATERIALS, METHODS AND SUBJECTS

Procurement of Normal Subjects. The original intention was that the old men to be used in this study would be selected from a home for retired sailors, but this institution, like other homes for the aged, was found to house many ailing men who had been admitted because of chronic illness and inability to receive adequate care at home. There seemed to be no satisfactory way to delineate in this population a group which would be a true sample of ageing males and not one loaded with those chronically ill, for example, with men partially disabled by cardiac decompensation or arthritis.

An alternative which had seemed *a priori* to offer good possibilities was the choice of subjects from men employed in an industrial concern or department store. Preliminary inspection of the personnel in two large companies emphasized, however, the extent to which such subjects varied in their living conditions at home and in some instances even in their type of physical work. Because of provisions for retirement of men in the seventh decade of life few persons were available in the older groups.

A third source, a prison, appeared to provide the most satisfactory group of subjects. They lived under an identical regimen. They had not been institutionalized because of physical disability, but because at an earlier time they had disobeyed the law. Furthermore these men were free to devote the required seven to sixteen days apiece to this study. It is superfluous to a humorous degree to emphasize that the routine of life was regulated in a similar fashion for all the men under study. Moreover, the use of such a group avoids the errors inherent in comparisons of age groups selected from different communities, such as would occur in contrasts of young men who are medical students with old men who live in a home for the aged. To be sure, the prisoners are obviously not a cross-section of the population of an area, but the men in different age groups were sufficiently similar as a group to permit comparisons. The prison in which these studies were carried out is a large one in the city of New York. Its inmates were committed for sentences usually of less than three years for crimes such as embezzling, petty larceny, and picking pockets.

Precautions were taken to avoid weighting of the group of subjects with certain types of men who are present in large numbers in prisons. Omitted from this study were homosexuals, narcotic addicts, and those confined to the hospital of the prison or with known histories of tuberculosis or untreated lues. In an attempt to insure further uniformity the survey was restricted to white men.

After observing the above precautions the men were chosen at random simply by drawing names from the list of prisoners in each age group. This was done to avoid selection or rejection on the basis of appearance or

past experience with the men. These men were then informed of the purpose of the study and allowed to accept or reject the invitation to serve as subjects. More than 95 per cent of the men chose to participate, partly from boredom perhaps and partly because of a desire to obtain a thorough medical examination.

The men were quartered in a separate section of the prison while under study, and with one exception cooperated wholeheartedly in the investigation. During the period when they were under study they did not engage in physical activities or in the tasks ordinarily carried out as part of the routine of the prison.

The range in age of the subjects was 21 to 75 years. Eleven men were in the third decade of life, 9 in the fourth, 11 in the fifth, 10 in the sixth, 7 in the seventh, and 3 in the eighth.

Examination of Subjects. Thorough physical examinations of each person were made separately by two of the group of investigators. In some instances the medical history obtained from each subject was supplemented from the files of the prison and hospital. Laboratory work consisted of hematological studies, including total and differential counts of blood corpuscles, hematocrit, corpuscular constants, hemoglobin level and sedimentation rate; chemical studies of the blood; routine urinalysis; an extensive roentgenographic investigation; a limited series of anthropological measurements, including specific gravity of the body and standardized photographic records; and a battery of common and uncommon measurements of the morphological and physiological status of each subject, especially of tests pertaining to the degree of development of various secondary sexual characters and of almost all items known or believed to be influenced by sex hormones. In selected subjects, special surveys were made of the semen and the cardiovascular status; the condition of the prostate as judged by rectal palpation; cytological study of prostatic smears; and histological examination of testicular biopsies. The following descriptions are restricted to procedures used in obtaining data related to the titers of ketosteroids in the urine.

Collection of Urine. Urine for the study of ketosteroids was collected for three to six days. The exact number of days of collection for each man is stated in Table 1. All samples of urine were obtained over consecutive periods of hours and days. As other means of disposal were not provided, no urine was lost inadvertently. Small individual containers were kept in the cell of each prisoner where they were available at all times. At intervals of six hours these bottles were emptied into five-gallon carboys containing 30 per cent sulfuric acid in amounts sufficient to maintain the urine at a pH of 4 or less in order to prevent ammoniacal decay. At the end of each period of collection the amount of acid was adjusted until it

TABLE 1. DATA FOR EACH MAN PERTAINING TO HIS AGE, THE INTERVAL DURING WHICH URINE WAS COLLECTED, AND THE VALUES FOR TOTAL KETOSTEROIDS AND FOR ALPHA- AND BETA-KETOSTEROIDS.

Case number	Age in years	Consecutive hours of urine collection	Ketosteroids (mg./24 hours)				Alpha-ketosteroids as % of total sum of ketosteroids	Beta-ketosteroids as % of total sum of ketosteroids	Ratio of beta- to alpha-ketosteroids
			*Total (before fractionation of crude neutral fraction)	Total (sum of alpha- and beta-ketosteroids)	Alpha	Beta			
1	21	84	14.0	16.0	15.1	0.9	94.5	5.5	5.9
2	22	84	13.7	14.1	12.7	1.4	90.0	10.0	11.0
3	23	144	10.4	11.7	9.1	2.6	77.7	22.3	28.6
4	23	144	6.8	7.0	6.9	0.1	98.8	1.2	1.5
5	23	84	15.5	15.5	14.5	1.0	93.5	6.5	6.9
6	23	120	7.2	7.2	—	—	—	—	—
7	25	84	11.5	11.9	11.6	0.3	97.5	2.5	2.6
8	27	120	10.5	11.5	11.0	0.5	95.6	4.4	4.5
9	27	72	6.1	6.5	5.7	0.8	87.7	12.3	14.3
10	28	120	8.1	8.6	6.0	2.6	69.7	30.3	43.3
11	29	144	8.3	9.2	8.8	0.4	95.7	4.3	4.5
12	30	120	8.2	8.6	6.7	1.9	79.0	21.0	23.4
13	32	120	10.1	10.1	—	—	—	—	—
14	33	144	6.9	7.0	5.9	1.1	84.3	15.7	18.7
15	33	84	15.0	14.8	14.1	0.7	95.3	4.7	5.0
16	33	84	20.5	20.0	18.1	1.9	90.6	9.4	10.5
17	34	84	9.5	10.2	9.2	1.0	90.1	9.9	19.9
18	35	144	5.6	5.2	4.2	1.0	80.7	19.3	23.8
19	38	120	10.6	11.7	9.0	2.7	76.9	23.1	30.0
20	39	144	9.1	9.4	8.9	0.5	94.7	5.3	5.6
21	40	120	6.2	6.0	4.6	1.4	76.7	23.3	32.0
22	41	120	7.8	7.8	6.8	1.0	87.2	12.8	14.7
23	44	84	14.4	14.1	12.7	1.4	90.0	10.0	11.0
24	45	72	4.7	4.9	4.9	<0.1	100.0	0.0	0.0
25	46	144	5.5	5.2	5.1	0.1	98.1	1.9	2.0
26	46	120	11.0	11.0	7.9	3.1	72.1	27.9	39.2
27	46	120	8.1	8.1	—	—	—	—	—
28	47	84	5.9	8.4	8.0	0.4	95.2	4.8	5.0
29	48	120	4.9	5.9	5.2	0.7	87.7	12.3	14.0
30	49	84	8.0	8.9	8.3	0.6	93.2	6.8	7.2
31	49	48	4.5	4.8	4.7	0.1	97.9	2.1	2.2
32	51	144	4.3	5.2	5.1	0.1	98.3	1.7	2.0
33	52	84	10.5	10.7	10.1	0.6	94.5	5.5	5.9
34	53	120	7.8	8.1	7.5	0.6	92.7	7.3	8.0
35	54	120	5.1	6.1	5.7	0.4	94.6	5.4	5.5
36	54	84	8.7	8.9	7.6	1.3	86.0	14.0	16.7
37	55	120	3.9	3.9	3.9	<0.1	100.0	0.0	0.0
38	55	120	4.0	4.4	3.9	0.5	88.6	11.4	13.9
39	57	48	2.2	3.6	2.7	0.9	74.5	25.5	34.2
40	58	120	4.0	4.5	4.1	0.4	92.3	7.7	8.2
41	59	120	4.4	4.8	4.7	0.1	98.0	2.0	2.1
42	60	120	6.6	6.3	6.1	0.2	96.3	3.7	3.9
43	60	120	4.1	3.8	3.5	0.3	91.6	8.4	8.6
44	60	120	3.6	3.8	3.7	0.1	97.5	2.5	2.7
45	62	120	3.1	3.9	3.3	0.6	84.6	15.4	18.4
46	63	48	3.0	4.0	3.6	0.4	89.8	10.2	11.5
47	65	84	5.9	6.9	6.8	0.1	98.5	1.5	1.5
48	68	48	4.6	4.6	4.6	<0.1	100.0	0.0	0.0
49	70	48	2.2	2.1	2.1	<0.1	100.0	0.0	0.0
50	72	144	3.9	4.0	4.0	<0.1	100.0	0.0	0.0
51	75	144	2.4	2.7	2.3	0.4	85.0	15.0	17.6

* The values for total ketosteroids found in the fourth column are those obtained by colorimetric determination of the urinary extract before fractionation, while those in the fifth column represent the sum of the values obtained from direct measurement of the alpha- and beta-ketosteroids.

represented 10 per cent by volume of the total quantity of urine. The urine was extracted within three weeks after collection; collections were made between July and September of 1947.

In the instances listed in Table 2 a second sample of urine was obtained after an interval of several weeks. This was done to check the validity of

TABLE 2. SIMILARITY OF VALUES FOR ORIGINAL AND DUPLICATE COLLECTIONS OF URINE FROM A GROUP OF 12 CASES CHOSEN AT RANDOM.

Case number	Age in years	Number of hours of urine collection	Ketosteroids (mg./24 hours)		Difference (in mg.) between original and duplicate samples
			second sample*	original sample	
3	23	48	11.2	11.7	+0.5
8	27	48	8.8	11.5	−2.7
18	35	72	5.2	5.2	0.0
21	40	48	4.8	6.0	−1.2
38	55	72	5.6	4.4	+1.8
40	58	72	6.4	4.5	+1.9
42	60	72	6.0	6.3	−0.3
43	60	48	4.5	3.8	+0.7
44	60	72	3.8	3.8	0.0
45	62	72	3.7	3.9	−0.2
50	72	48	4.2	4.0	+0.2
51	75	72	2.4	2.7	−0.3

* The interval between the collection of the first and second samples of urine varied from 2 to 8 weeks.

determinations. After it became apparent that the values for many of the prisoners were lower than those for some of the personnel of the medical laboratories and hospital, additional studies were made at intervals in laboratory personnel, thus making certain that the method of extraction and assay continued to give such high values. The ages of the persons not in prison and data pertaining to their titers of urinary steroids are given in Table 6.

Extraction, Separation and Measurement of Ketosteroids in the Urine. Extraction and separation of the ketosteroids followed closely the procedure utilized by Dobriner and associates (3) and colorimetric measurement was done by the method of Zimmermann (4) as modified by Holtorff and Koch (5).

In the calculations the term "total amount of ketosteroids" refers to the sum of the values for alpha- and beta-ketosteroids and not to data obtained by colorimetric examination of the neutral ketonic fraction. Colorimetric measurements of the neutral ketonic fraction were also made, and as can be seen from their tabulation in Table 1 they differ slightly in

TABLE 3. THE AVERAGE DAILY EXCRETION OF TOTAL KETOSTEROIDS SHOWS A PROGRESSIVE DECREASE WITH EACH DECADE OF ADULT LIFE.

Age in years		Number of men in group	Total ketosteroids (mg./24 hours)	
Range	Average*		Average	Range
20–29	24.6	11	10.8	5.9–15.5
30–39	34.1	9	10.1	5.2–20.0
40–49	43.6	11	7.7	4.8–14.1
50–59	54.8	10	6.0	3.6–10.7
60–69	62.1	7	4.8	3.8– 6.9
70–75	72.3	3	2.9	2.1– 4.0

* The averages per decade are computed from individual values listed in column 5 of Table 1.

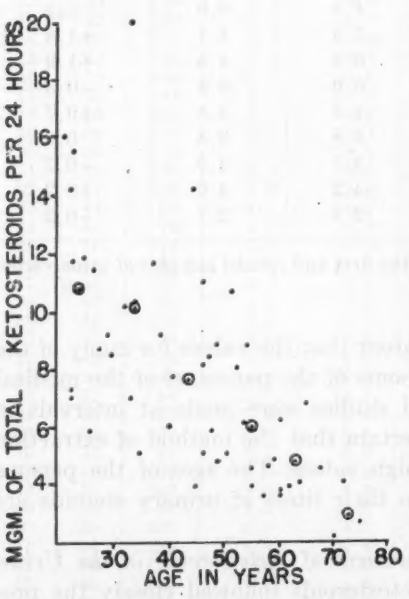

FIG. 1. Progressive decrease with age in the excretion of urinary ketosteroids. Dots indicate the titer for each man, circles the average for the men in that decade of life.

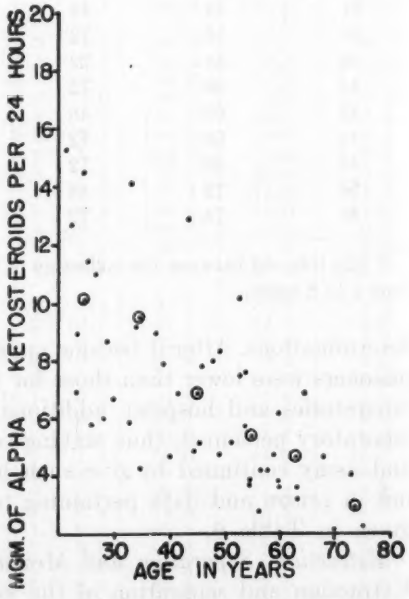

FIG. 2. Showing a decrease with age in average daily excretion of alpha-ketosteroids almost parallel with the decrease with age in the total excretion of ketosteroids, and accounting for much of this reduction.

many instances from the sum of the values for the alpha and beta fractions that were obtained after further purification.

It is to be noted that the values for alpha- and beta-ketosteroids are obtained by direct measurements of each fraction, whereas others (6, 7) have measured one fraction and calculated the other by subtraction from the value for the ketonic fraction. The beta fraction refers to hydroxy substances precipitated upon addition of digitonin to the neutral ketonic fraction, and the alpha fraction consists of those hydroxy compounds that do not precipitate when treated with digitonin.

RESULTS

Accuracy of Methods. A check on the possibility of error in the collection and preservation of urine and in the accuracy of the measurements of the

TABLE 4. PROGRESSIVE DECREASE OF THE AVERAGE EXCRETION OF ALPHA- AND BETA-KETOSTEROIDS IN THE SUCCESSIVE DECADES OF LIFE (COLUMNS 4 AND 6) AND THE RATIOS OF THE BETA- TO TOTAL, AND BETA- TO ALPHA-KETOSTEROIDS (COLUMNS 9 AND 10).

Age in years		Number of men in group	Ketosteroids (mg./24 hours)				Ratio of		
			Alpha-		Beta-		Alpha- to total ketosteroid	Beta- to total ketosteroid	Beta- to alpha-ketosteroid
Range	Average		Average	Range	Average	Range			
20–29	24.8	10	10.1	5.7–15.1	1.1	0.1–2.6	91.0	9.0	12.3
30–39	34.3	8	9.5	4.2–18.1	1.3	0.7–2.7	86.3	13.7	16.5
40–49	45.5	10	6.8	4.7–12.7	0.9	0.1–3.1	90.2	9.8	12.7
50–59	54.8	10	5.5	2.7–10.1	0.5	0.1–1.3	92.0	8.2	9.7
60–69	62.1	7	4.6	3.3– 6.1	0.2	0.1–0.3	94.0	6.0	6.7
70–75	72.3	3	2.8	2.1– 4.0	0.1	0.1–0.4	95.0	5.0	5.9

ketosteroids was provided by assays on duplicate collections of urine from a few men chosen at random, except that most of them were in the later years of life where study seemed needed most. Agreement between the values for the first collection of urine and those of the second determination was fairly close as shown in Table 2. The greatest differences in any of the cases are less than the expected variation from day to day in normal men, which may amount to as much as 5 milligrams (8, 9, 10, 11).

General Aspects of Data. The total amount and the quantities of alpha- and beta-ketosteroids excreted per twenty-four hours are listed for each patient in Table 1. Tables 3 and 4 present the average and range values for each decade of life. Figures 1, 2, and 3 show the values for each individual in black dots and the average excretion for the men in each decade of life by dots within circles.

From these tables and graphs it can be seen that in all three categories of steroids, that is, in the alpha- and beta-ketosteroids and in the sum of these fractions, the average titers declined with age in a progressive and pronounced fashion. The excretion of total and alpha-ketosteroids diminishes with each decade. The average values for the beta-ketosteroids decrease similarly except for a peak in the fourth decade. This exception appears to be a fortuitous one to be expected when small segments of the

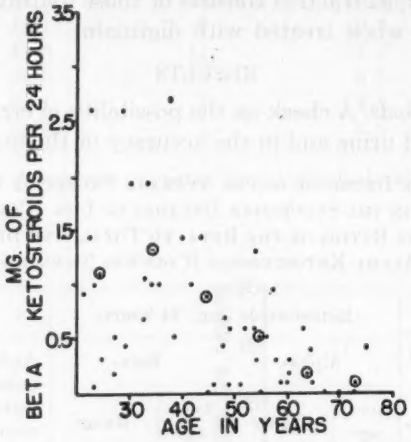

FIG. 3. The excretion of beta-ketosteroids also decreases with age.

data are considered; it is related to the absence in this decade of very low values, several of which are found in the preceding and in all succeeding decades.

Values for Sum of Alpha- and Beta-ketosteroids. The decline in values for the average daily excretion of total ketosteroids follows almost a straight line decade by decade. In comparison with the output in the third decade, that in the seventh decade is less than one-half and that in the eighth decade only 27 per cent (Table 3).

Values for Alpha-ketosteroids. The fall in total output of ketosteroids is due mostly to a loss in alpha-ketosteroids which comprise on the average more than 90 per cent of the ketosteroids. Therefore, the curve of decline in alpha-ketosteroids (Fig. 2) follows closely that of the total ketosteroids (Fig. 1). In comparison with the average in the third decade, the excretion in the seventh decade was more than halved and that in the eight decade reduced to 28 per cent (Table 4).

Values for Beta-ketosteroids. With the passing years the excretion of

beta-ketosteroids diminishes (Fig. 3) in much the same fashion as that of alpha-ketosteroids and the sum of these two fractions. An exception to this generalization is the average value for the fourth decade which may not be truly representative of this age because of reasons stated before. In terms of percentage decrease from the average value in the third decade, that in the sixth decade was less than half, that in the seventh less than one-fifth, and that in the eighth decade only 9 per cent.

Variability from individual to individual was the rule as regards the proportion of beta compounds in the total ketosteroid ouptut (Fig. 4). It was

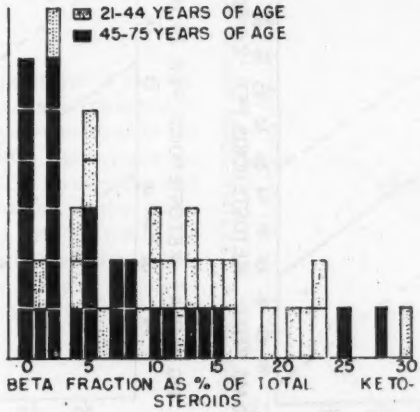

FIG. 4. Graphic portrayal of the effect of ageing upon the percentage of ketosteroids excreted as beta compounds. Each block represents the total for one man.

less than 5 per cent of the total in 31 per cent of the entire group of men; from 5 to 10 per cent in 34 per cent of the men; and above 10 per cent in the remaining 35 per cent.

The percentage of the total ketosteroids formed by the beta fraction appears to be related to age when the data are considered in averages by decades (Table 4), or in two larger groups of men, 21 to 44 and 45 to 75 years of age. Less than 5 per cent of the ketosteroids were excreted as beta compounds in 19 per cent of the men who were 21 to 44 years old but in 44 per cent of those 45 to 75 years old. Of the 13 men in the entire series whose beta fractions were so low as to amount to no more than 2 per cent of their total ketosteroids, 11 were 45 or more years of age. Expressed as percentages, 41 per cent of the men over 45 years of age had only 2 per

cent or less of beta compounds whereas only 11 per cent of those 21 to 44 years old had such a small proportion of beta compounds (Fig. 4).

Conversely a beta fraction in excess of 15 per cent of the total output was observed in 42 per cent of those less than 45 years of age, in contrast to only 7 per cent of those 45 years of age or more.

In previous investigations (12, 13, 14) the proportion of beta-ketosteroids in the total output has not exceeded 15 per cent except in an occa-

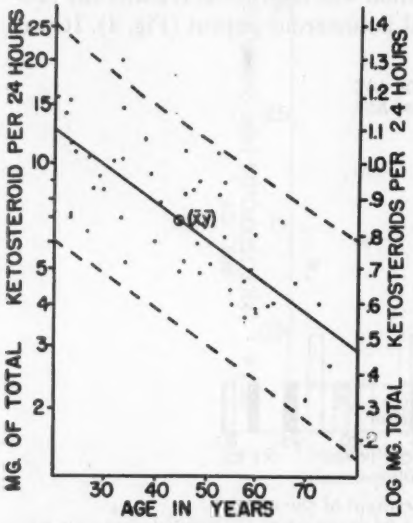

FIG. 5. Graphic representation of the values for total ketosteroid titers statistically analyzed and summarized in Table 5. The x coordinate has been plotted arithmetically, and the y coordinate logarithmically.*

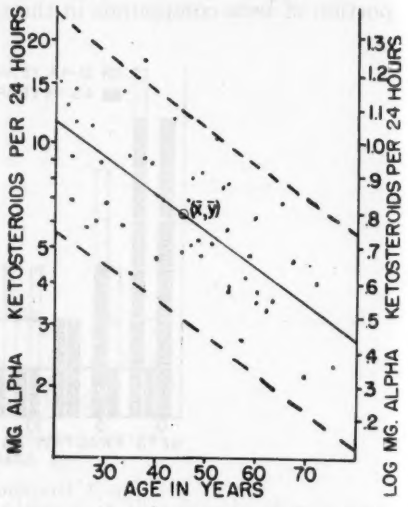

FIG. 6. Graphicre presentation of the statistical analysis of the values for alpha-ketosteroids.

* Conversion of ketosteroid values to logarithmic equivalents facilitates comparison of the curves for total, alpha- and beta-ketosteroid output or for titers obtained in individual men; comparisons can be made with other diverse measurements such as weight, height, and specific gravity of the subject, since the logarithmic equivalents are independent of units of mensuration; adjustment is made for the variation in output as a function of age, so that 2 instead of 3 variables remain for analysis. The line of regression was found in the usual manner by substituting values of x in the equation, solving for y, plotting these values, and drawing a curve through the points located. The points determining the zone of error are found by adding to and subtracting from various y values twice the standard error of estimate for those y values, plotting these points with respect to the corresponding x value, and drawing a curve through the points (broken lines on the graph). In a group of cases selected at random where distribution is normal, 95 per cent of such cases will fall within the zone of error. $(\bar{x}, \bar{y})$ on the graph represents the average age and ketosteroid titer for the entire group, i.e., the mean.

sional case (15), whereas eight such instances were found in the present series.

Statistical Analysis of the Entire Series. Since the total number of subjects in the study is relatively small it seemed desirable to analyze the data as a whole in addition to discussing the results by decades. The advantage in the former method is that errors in sampling are less likely to appear, but the latter method has its value here where the groupings are

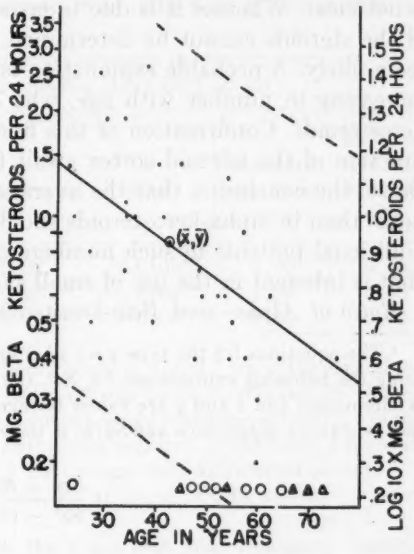

FIG. 7. Graphic portrayal of the statistical analysis of the beta-ketosteroids. A factor of 10 has been employed in the logarithmic scale for convenience in plotting the graph. The open circles and triangles at the bottom of the graph represent values that were left out of the calculations. The circles are for values of 0.1 mg. and the triangles for those values less than 0.1 mg.

so small that strict statistical analysis cannot be made. It should be noted that the chief difference in the data treated as a whole from that given simply as average figures by decades, is that in the latter method the very lowest values for the beta-ketosteroids are omitted since they seem to form a discontinuous series with respect to the remaining data for this fraction. Further, as will be seen later, when the very low figures are omitted from the calculations, the ratios of beta- to total and beta- to alpha-ketosteroids do not fall with increasing age as they do when analyzed by averages for each decade. This apparent discrepancy is explained, of course, by the fact that the figures in the later decades are weighted by the low titers obtained in several men. Figures 5, 6, and 7 show graphically the curves obtained by such statistical treatment of the data for the excretion of total, alpha- and beta-ketosteroids. Table 5 lists the values of the means, slopes, and intercepts for the equation of these curves as well as the standard errors, standard deviations, and standard

errors of estimate for the average total, alpha-, and beta-ketosteroid output.[4]

In comparing the three equations derived, it can be seen that the slopes are all very nearly equal which indicates that total, alpha-, and beta-ketosteroid outputs decline at similar rates as age increases, and that the ratios of alpha- to total, beta- to total, and beta- to alpha-ketosteroids are equal throughout life.

The significance of the apparent discontinuity of values discussed before is not clear. Whether it is due to errors in collection, extraction, or assay of the steroids cannot be determined, although these possibilities do not seem likely. A probable explanation is that there is a small group of men, increasing in number with age, who excrete very low amounts of beta-ketosteroids. Confirmation of this finding and its possible relationship to function of the adrenal cortex await further investigation. Whatever the cause, the conclusion that the average decrease with age is more rapid in beta- than in alpha-ketosteroids should await substantiation in studies of additional patients in such numbers as to exclude the possibility of error that is inherent in the use of small samplings.

Ratio of Alpha- and Beta-ketosteroids. The proportion of the total ke-

[4] The equations (of the type $y = a + bx$) are derived by the method of mean squares using the following expressions: Sx, Sx^2, $(Sx)^2$, Sy, Sy^2, $(Sy)^2$, Sxy, and $SxSy$, where S is summation and x and y are values for age and ketosteroid output respectively. The means, $\bar{x}$ and $\bar{y}$, equal Sx/n and Sy/n; a, the y intercept, equals $y - b\bar{x}$; b, the slope, equals

$$\frac{Sxy - SxSy/n}{Sx^2 - (Sx)^2/n}.$$

The standard error for $\bar{y}$ is

$$\frac{\text{Standard Deviation}}{\sqrt{n}}.$$

Where $[y^2] = Sy^2 - (Sy)^2/n$, $[xy] = Sxy - SxSy/n$ and $[x^2] = Sx^2 - (Sx)^2/n$, other quantities are derived as follows: the standard deviation for $\bar{y}$ is

$$\sqrt{\frac{[y^2]}{(n-1)n}}$$

and the standard error of estimate is

$$\sqrt{\frac{[y^2] - [xy]^2/[x^2]}{n-2}}.$$

tosteroid output represented separately by the alpha and beta fractions, and the relative proportions of these two fractions one to the other, are tabulated in Table 4 for data arranged by decades.

The average decrease with age in values for beta-ketosteroids is more pronounced than the reduction in total ketosteroids even though the decrease in the total ketosteroids by the eighth decade amounted to about three-fourths of the output in the third decade. The average excretion of beta-ketosteroids, which constituted about 10 per cent of the total quantity of ketosteroids in the third decade, was only 5 to 6 per cent in the

TABLE 5. SUMMATION OF THE VALUES OBTAINED IN THE STATISTICAL ANALYSIS OF THE EXPERIMENTAL DATA OF TABLE 1.

Ketosteroid	$\bar{x}$ Average age (years)	$\bar{y}$ Average titer in mg./24 hours	a y intercept	b slope	Standard deviation of y	Standard error of estimate of y
Total	44.75	6.98 ± 0.15	2.32	-0.0106**	1.07	0.15
Alpha	45.44	6.28 ± 0.16	2.27	-0.0104**	1.08	0.15
Beta*	42.17	0.93 ± 0.06	1.38	-0.0106**	0.35	0.30

The equations for the lines of the graphs in Figures 5, 6, and 7 are of the general form for linear regression: $y = a + bx$, where x and y are the linear coordinates (in this case representing age and log mg. of ketosteroid output per 24 hours respectively), a is the y intercept, and b is the slope of the curve, in this case negative. In the table, $\bar{x}$ represents the average age for the entire group and $\bar{y}$ the average daily ketosteroid output.

* 12 values omitted (cases 4, 24, 25, 31, 32, 37, 41, 44, 47, 48, 49, and 50) for reasons given elsewhere.

** Significantly different from zero at the 1 per cent level (Snedecor, *Statistical Methods*, 1946, Chapter 6 and Table 10.7).

seventh and eighth decades. Because of the greater average decline in beta- than in alpha-ketosteroids the ratio of the beta to the alpha compounds seems to decrease slightly in the later years (Table 4).

Relationship between Ketosteroid Values and the Mass of Body Protoplasm. An analysis still in progress shows a high degree of correlation between the quantities of ketosteroids in the urine and the weights of the individual men. In every decade the output of ketosteroids was less, the lighter the men. Among the men under study, who had as a group very little tendency to obesity, the decline in values with age was definitely associated with a decrease in body weight. More detailed consideration of the relationship between titers of urinary ketosteroids and body weight is reserved for future consideration which will also include height, specific gravity, surface area and other pertinent features.

Individual Variability in Values of Ketosteroids. There is great variance in values from individual to individual, as has been reported previously (8, 10, 11, 12, 14–26).

DISCUSSION

Several points are worth noting in comparing the results in the present survey with observations made by other workers. The values for the men in this series are slightly lower than some of those reported by other investigators, most of whom have dealt with more restricted age groups, particularly with young men in the third and fourth decades of life. This difference between our results and those of others does not appear to be due to a loss of ketosteroids in the present study, since the results obtained with our methods on the urine of normal young adult males living outside the prison yielded values comparable with those of other workers. These data, obtained on the personnel of the medical laboratory and hospital, are shown in Table 6.

TABLE 6. VALUES FOUND IN NORMAL PERSONNEL OF THE LABORATORY AND HOSPITAL AS REGARDS THE AVERAGE DAILY EXCRETION OF URINARY KETOSTEROIDS.

Case	Age in years	Number of hours of urine collection	Ketosteroids (mg./24 hours)
A	18	72	15.1
B	18	96	17.1
C	24	96	10.0
D	25	120	10.5
E	26	96	18.0
F	27	96	17.2
G	30	72	21.0
H	47	48	10.4

One explanation for the difference between the data of prisoners and those of other subjects may be the fact that both the food and routine of life in the prison were unpalatable to the prisoners. With few exceptions the men disliked the diet and refused portions of each meal. Many complained that they were always hungry, and it was the usual impression of most prisoners that they had lost weight during imprisonment.

It should be noted too that the physical activities of the men were slight during the period of study.

Another matter to be considered is the possibility that the length of stay in prison might be correlated with the titers of ketosteroids. Figure 8 shows that there is no such relationship between these items. Not elimi-

nated is the likelihood that many of these men were not in good health even prior to commitment in prison. Some had been jailed as a result of sequelae of bouts of alcoholism and a few had been hiding from the law. It is possible, then, that a lack of well-being involving a loss of weight in several instances may explain in part the somewhat lower values than those usually reported in young men.

It has been found (17, 18, 19, 27, 28, 29) that in various diseases and instances of poor health the output of urinary ketosteroids may be lowered considerably. The subjects of this study, with a few exceptions, cannot be considered to be in ill health. All were participating actively in routine prison affairs prior to the beginning of the survey. Table 7 shows pertinent clinical findings in those members of the group who might be considered

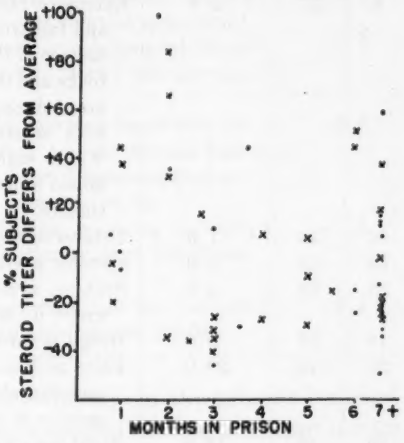

FIG. 8. Showing that there is no correlation between the length of each subject's internment in prison and the percentage by which his titers of total urinary ketosteroids differ from the average for the men of his age. The data for the younger men are represented separately by crosses in order to distinguish them from the values for older men; crosses represent those subjects less than 50 years of age.

to be below par in health. No disease was observed in the majority of the men in the third decade, that is, in the younger men for whom higher values might have been expected; the titers of 2 men with somewhat abnormal clinical findings did not differ much from the average for the decade. Similarly, examination of the right-hand column in Table 7 shows that the values for ketosteroids did not tend to be less than the average for the decade in men of other ages in whom one or another abnormality was detected in the medical examination. This is evidence that the low titers in these groups did not arise from illnesses of certain men.

Another matter to be considered and then disregarded in this connection is the definitely lower values in specimens collected in winter as compared to those obtained in summer (18). All collections for this study were made during summer months.

With regard to claims that a climacteric-like state occurs in men, it

TABLE 7. STATUS OF THE MEN WHO DESERVED THE MOST CONSIDERATION AS TO WHETHER OF NOT THEY WERE POSSIBLY OR ACTUALLY ILL. THE TITERS OF KETOSTEROIDS IN THESE CASES DO NOT DIFFER APPRECIABLY FROM THE AVERAGE FOR THEIR DECADE.

Case	Age in years	Sedimentation rate in mm./hour (Westergren)	Medical Status	Difference between ketosteroid titer and average for age group (mg./day)
2	22	—	Controlled diabetic.	+3.3
6	23	2.8	Extensive rash on flexor surface of arms and forearms, on popliteal spaces, inner surface of thighs, inferior aspect of buttocks and dorsum of neck. Skin of these areas thickened, encrusted and scaling, with crusts raised from skin surface. Where scales flaked off, area was reddened and fluid oozed from the naked surface.	−3.6
12	30	7.0	Extensive acne on back.	−1.6
15	33	2.0	Chronic postnasal drip.	+4.7
16	33	5.0	Scabies; systolic murmur judged to be grade ii; extrasystoles.	+9.9
18	35	5.0	Irregular cardiac rhythm (not fibrillating).	−4.9
26	46	29.0	Faint pink macular rash on trunk with no papular elevation but otherwise suggestive of hives.	+3.3
29	48	12.0	Right leg amputated as a result of trolley accident. Many bones broken at this time.	−1.8
30	49	27.0	Scoliosis; dribbling of urine (collection	+1.2
33	52	2.5	bag). Right hydrocele tapped repeatedly about 10 years ago.	+4.7
35	54	13.0	Pain in shoulder joints on movement of arms above horizontal position; pain to deep palpation over deltoid areas; no abnormalities visible by x-ray.	+0.1
39	57	—	Soft penile chancre; treated for lues 28 years ago for a period of 16 months.	−2.4
41	59	54.4	Varicose ulcers on right lower leg.	−1.2
42	60	27.0	Mild cardiac decompensation.	+1.5
43	60	24.0	Healed varicose ulcers of legs.	−1.0
44	60	31.0	Left leg amputated following extensive varicose ulceration; varicose ulcers on right lower leg.	−1.0

TABLE 7 (Continued)

Case	Age in years	Sedimentation rate in mm./hour (Westergren)	Medical Status	Difference between ketosteroid titer and average for age group (mg./day)
45	62	35.0	Chronic hypertrophic arthritis for many years.	−0.9
46	63	—	Extreme fungus infection of left foot; foot swollen to twice normal size.	−0.8
47	65	28.5	Diarrhea of unknown etiology for few days prior to participation in survey.	+2.1
48	68	—	Questionable cardiac enlargement; blood pressure 190/80; ?aortic valvular disease.	−0.2
49	70	—	Marked systolic murmur heard over entire precordium and all normal heart sounds absent; no decompensation evident; blood pressure 150/90.	−0.8
50	72	11.0	Auricular fibrillation; receiving digitalis; no decompensation evident; blood pressure 160/80.	+1.1
51	75	41.0	Operation some years ago for varicose ulcers of leg: blood pressure 160/80.	−0.2

should be noted that the values for ketosteroids in the present series of men indicate that a gradual rather than a sudden decline of output occurs with ageing. Until information is obtained as to the excretion of gonadotropins and androgens by these subjects the data at hand are insufficient to allow definitive statements.

The literature seems to contain no survey of the excretion of ketosteroids in the later decades of life or of the effect of ageing upon those values. Venning and Kazmin (10) in a study of 14 males ranging in age from 20 to 51 years reported the average excretion of 16.6 mg. of ketosteroids per twenty-four hours, with a range of 10 to 25 mg.; two other men aged 77 and 81 excreted 10.0 and 10.6 mg. per twenty-four hours. These figures do not appear to be significantly less than their findings in the younger men. Similarly, Luft's (21) series, which contains 15 subjects under 40 and 8 subjects over 40 years of age, shows no significant falling off of values with increased age. The lowest titers were found in the 2 youngest and 2 oldest subjects; the values for these 4 persons aged 16, 22, 71, and 82 years were 15.5, 19.8, 14.3, and 18.6 mg. respectively, considerably less

than the average of 26.2 mg. for the entire group. In Salter's (15) series of normal males, only two are past the fourth decade; and the values for these, aged 42 to 58 years, are 23 and 12 mg., compared to an over-all average of 15.3 mg. for 15 subjects. As each of these studies contains comparatively few subjects in the decades beyond the fourth, it is doubtful whether one is justified in drawing conclusions from them. In the investigation carried out by Chou and Wang (17) there is a decline in ketosteroid values with increasing age, but here again there are only 4 men in the fifth, 2 in the sixth, and 2 in the seventh decades. Wooster (25) investigated the titers of ketosteroids in a group of 49 subjects whose range of age was the third to sixth decades. The mean values for one decade compared to another were not significantly different in his series, although he noted that in terms of milligrams the variation in output from individual to individual tended to decrease with age.

Data in the literature that bear upon the relation of age to alpha- and beta-ketosteroids are even more limited than those pertaining to the total output. Most studies of alpha- and beta-ketosteroids have been made with reference to their excretion in disease (6, 23, 30, 31) particularly in hyperplasia or neoplasia of the adrenal cortex, whereas the present study is concerned with the amounts of each fraction excreted by normal individuals and the relations of such values to the endocrine state in later life. The findings in the present study would appear to be at variance with the data of Salter (15), in that with ageing a) the change in the excretion of alpha compounds more or less parallels the decline in values of total ketosteroids (Tables 3 and 4; Figs. 1 and 2); and b) the average beta values also tend to decline in amount with increasing years (Tables 3 and 4; Figs. 1 and 3). As there are only 2 subjects over 40 years of age in Salter's series, valid comparisons cannot be made with his data.

Finally, it should be noted that as the titers of total ketosteroids decrease with age there is no great relative increase in the amount of either alpha or beta compounds that would suggest compensatory function by the adrenal cortices in response to any loss of testicular function.

SUMMARY

The effect of ageing upon the excretion of urinary ketosteroids and upon the respective amounts of the alpha- and beta-ketosteroids has been studied in a group of 51 apparently normal men, 21 to 75 years of age, all of whom were living under similar conditions. In successive decades of life the average daily excretion of ketosteroids decreased progressively. The material extent of this reduction in output is shown by the fact that in comparison with the quantity present in the third decade, that present in the seventh decade was less than half and that in the eighth, only 27 per cent.

The major part of this decline is due to a loss of alpha-ketosteroids which accounts in general for over 90 per cent of the total quantity of urinary ketosteroids. The curve of decrease in alpha-ketosteroids parallels closely that for the total output of ketosteroids.

The excretion of the beta-ketosteroids also decreased with age. Very low values were uncommon in the early years of life but frequent in men in the sixth and seventh decades.

In this series of men, who had very little tendency to obesity as a group, the output of ketosteroids in all decades of life was correlated with the body weight of the individual man.

ACKNOWLEDGMENT

The authors are indebted to Dr. Peter Amorosa, late Commissioner of Crime in New York City; Dr. Herman Baxt, resident physician; Warden Johnson, and the men who were subjects in this study at Riker's Island Penitentiary. We wish also to acknowledge gratefully the aid of three medical students, Jacob Katz, Irwin Katzka and Gerald Rodman; the advice of Dr. Konrad Dobriner as to the procedures used in the preservation of urine and the extraction and assay of ketosteroids; and the help in statistical treatment of the data by Drs. Robert Lehman and John Tukey.

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EXPERIMENTAL ALTERATION OF THE HUMAN OVARIAN CYCLE BY ESTROGEN*

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THERE are relatively few studies of the effect of estrogen on the human ovarian cycle. Sturgis and Albright (1) and Sturgis and Meigs (2) demonstrated that ovulation was inhibited in women when estrogen was administered in the early part of the menstrual cycle. This inhibition evidently depended upon early administration of the estrogen (before the sixth day) as well as upon the dosage employed. Their primary interest was in the relief of dysmenorrhea which was obtained by eliminating the luteal phase from the cycle.

They found that bleeding usually occurred from twelve to fourteen days following a single 10 mg. dose of estradiol dipropionate. However, if the dose were repeated in ten days the expected bleeding would be delayed and by suitable timing of the estrogen administration, bleeding could be delayed for as long as three months. Thus, by alternating intervals of ten or fourteen days between doses, it was possible to bring about bleeding at predictable intervals of two weeks, four weeks or eight weeks.

Gillman (3) described the effect of single doses of estradiol benzoate on the length of the human menstrual cycle. He found that small doses (.01 to 0.5 mg.) had little effect but when a dose of 5 mg. was given on the eighth day of the cycle there was an inconsistent effect, some cycles being shortened to nineteen days and others lengthened to forty-five days. Gillman did not report any endometrial biopsies to explain how the ovarian cycle had been affected. Fluhmann and Hoffman (4) failed to produce any alterations in the cycle with 0.05 to 0.10 mg. of estrogen daily. Westman (5) claimed that estrogenic therapy favored luteal function but his evidence was equivocal.

The present study was undertaken to determine in what manner the human cycle is altered by the administration of estrogen. Information relative to the ovarian cycle was obtained by weekly endometrial biopsies.

METHODS

Each of the women selected for this study presented a history of a nor-

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mal menstrual rhythm. On most of the subjects[1] it was possible to obtain weekly endometrial biopsies for consecutive months, before, during, and after treatment. Some of the patients were seeking relief from dysmenorrhea. Stilbestrol was the estrogen used and it was given orally according to three general schedules: Early in the cycle during the proliferative phase; late in the cycle during the luteal phase; and continuously during the cycle.

EXPERIMENTAL RESULTS

EARLY TREATMENT

Six patients were given a single 20 mg. dose of stilbestrol early in the menstrual cycle (4th, 5th, or 6th day), as shown in Figure 1. The next menstrual period was delayed, the interval varying from thirty-three days for M. J. to forty-two days for M. W., with an average duration of thirty-eight days for the group. As indicated in the graph, the control menstrual intervals before and after treatment were normal and averaged thirty and twenty-nine days respectively.

Biopsies, taken after the estrogen treatment, revealed secretory endometrial changes in 5 of the 6 patients (M. J. being the exception). Prolongation of the menstrual interval was associated with a delay in the onset of the secretory pattern. The duration of the luteal phase at the end of the cycle was the same as that seen in a normal untreated cycle.

A similar study was made on 5 women who complained of dysmenorrhea (Fig. 2). The menstrual interval was prolonged from thirty-four to thirty-seven days, as compared to a normal twenty-eight to thirty day cycle. In 4 of these patients, the premenstrual biopsy showed secretory endometrium and the dysmenorrhea was not affected by the estrogen. One patient (M. S.) however, did have an anovulatory cycle following the 20 mg. dose of stilbestrol. In only two or three instances was there any nausea following the administration of the drug.

Seven to ten days after the stilbestrol was given, 5 of the patients in these two groups (Figs. 1 and 2) experienced slight vaginal bleeding that lasted from two to four days. Since the bleeding was so scanty and was not associated with changes in the endometrial pattern, it was considered as an estrogen-withdrawal bleeding rather than a menstrual period. In patient M. S. (Fig. 2) it recurred on the twenty-seventh and twenty-ninth days of the cycle. These episodes of slight bleeding (usually not enough to require a pad) may have been similar to those that led Gillman to state that the cycle was shortened in 2 of his cases. Four of Gillman's patients treated with 5.0 mg. doses of estradiol benzoate experienced cycles of thirty-one to

[1] Through the courtesy and cooperation of Doctor Adolph Soucek of Mount Pleasant State Hospital, patients were made available for these observations.

forty-five days, comparable to the majority of our cases. Sturgis and Meigs also found that a 10 mg. dose of estradiol dipropionate would prolong the interval. Patient No. 3 in their series experienced slight bleeding on the fifteenth day and then had a painful (ovulatory) menstrual period forty-six days after the previous normal menses.

THE EFFECT OF A SINGLE LARGE DOSE OF ESTROGEN GIVEN EARLY IN THE MENSTRUAL CYCLE (DELAY IN OVULATION)

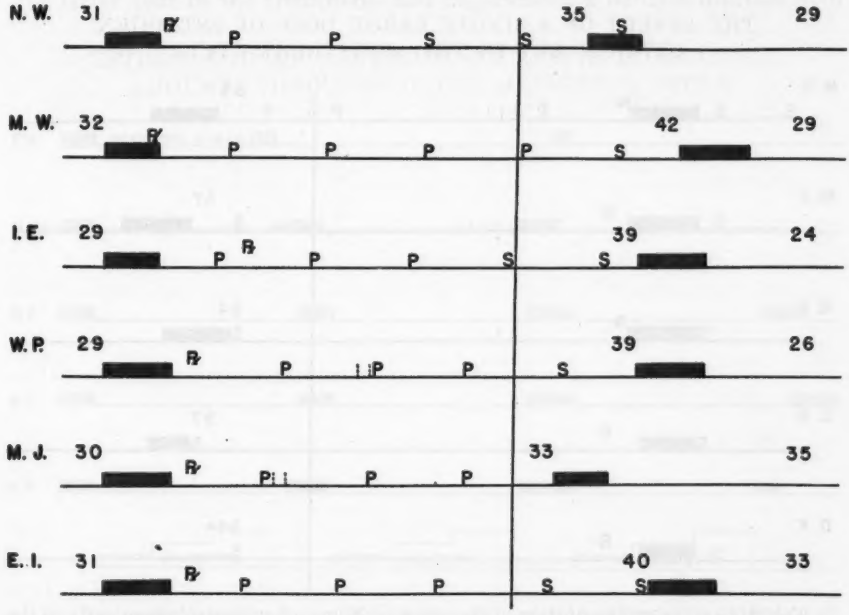

FIG. 1. Graphic representation of the effect of 20 mg. of stilbestrol given orally early in the cycle. The numbers represent the length of the cycle before, during and after treatment. The letters represent the stage of the endometrium, P for proliferative and S for secretory, as revealed by weekly biopsies. The vertical line is 30 days after the onset of the previous period. The dotted lines represent days of slight vaginal bleeding, whereas the menstrual periods are denoted as solid black bars.

Since the present findings are in apparent agreement with those of Sturgis and Meigs and of Gillman it may be stated that a single dose of estrogen given early in the menstrual cycle will usually delay but not inhibit ovulation and will thereby increase the length of the cycle.

PROLONGED TREATMENT

Sturgis and Albright (1) found that when estrogen was given continuously from the fourth or fifth day of the cycle until the eighteenth or twen-

tieth day, ovulation was prevented and the luteal phase of the endometrium did not develop. Under these conditions dysmenorrhea was completely relieved. Either estradiol benzoate 1.6 mg. intramuscularly every third day or oral stilbestrol 1 mg. daily was equally effective. Under these conditions of therapy they found that dysmenorrhea could be relieved (ovulation prevented) on alternate months but not in consecutive cycles. Randall and Odell (6) found that ovulation was inhibited and dysmenor-

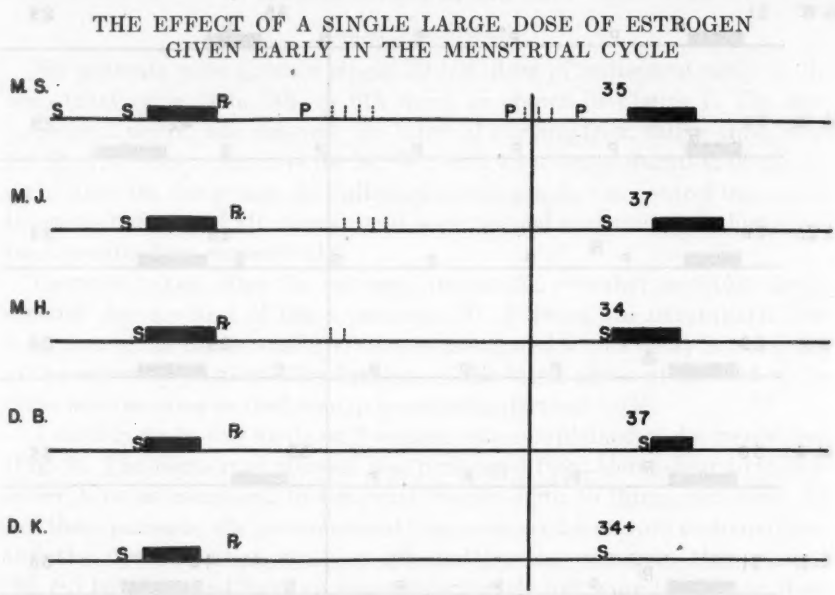

FIG. 2. Graphic representation of the effect of 20 mg. of stilbestrol given early in the cycle as a single oral dose. The vertical line is 28 days after the onset of the previous period. The rest of the graph is the same described for Figure 1. Biopsies were obtained only in the premenstrual period.

rhea relieved when 1 mg. of stilbestrol was given daily for twenty days following cessation of menstruation.

A group of patients was given stilbestrol to determine whether relief from dysmenorrhea could be obtained with an oral estrogen for three successive months. Starting at the end of a menstrual period, 1 mg. of stilbestrol was given daily for ten days, 2 mg. daily for the next ten days and 3 mg. daily for the third ten days. Bleeding usually occurred within three days after the last 3 mg. dose and the dysmenorrhea was completely relieved (Fig. 3). One attempt with hexestrol on a 3, 6 and 9 mg. increasing dose schedule was ineffective, whereas the patient obtained relief the fol-

lowing month with stilbestrol. Endometrial biopsies obtained in the third month of treatment in 3 of these patients revealed a proliferative endometrium, as would be expected from the findings of Sturgis and co-workers. There was no evidence of hyperplasia or more profuse bleeding in these patients followed for three successive months.

The side effects of stilbestrol were apparent in some instances. Two patients noticed a definite weight gain (5 to 11 pounds) and edema during the latter part of the treatment, and experienced a definite diuresis with

ESTROGEN GIVEN CONTINUOUSLY AND IN INCREASING AMOUNTS THROUGHOUT THE MENSTRUAL CYCLE

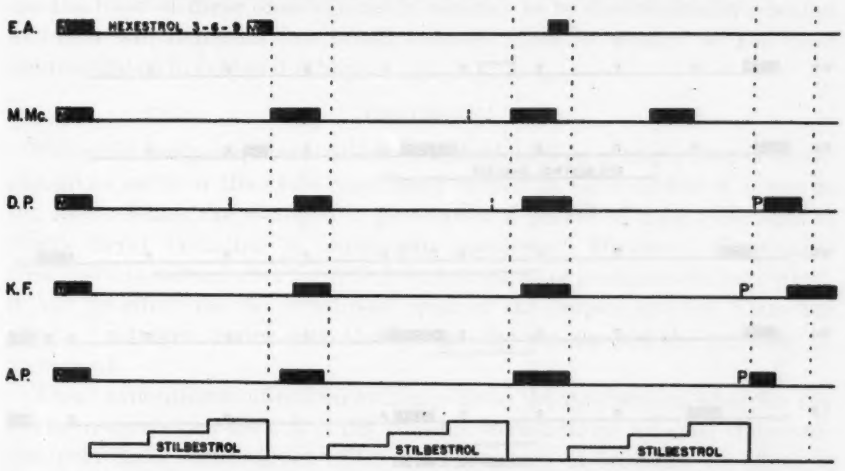

FIG. 3. Graphic representation of prolonged treatment with stilbestrol in patients with dysmenorrhea. The black bar with the zigzag represents a painful menstrual period, whereas, the other periods were painless. The daily dose of stilbestrol was increased at intervals of ten days from 1 to 2, and then to 3 mg. orally. The medication was withheld for ten days before the next series was offered. Proliferative endometrium (P) was obtained in three instances.

the onset of bleeding. One patient also experienced episodes of nausea and headaches during the treatment. A patient not shown on the chart discontinued therapy because of nausea. For those patients who tolerate the drug, stilbestrol can be given orally in such a manner that dysmenorrhea may be completely relieved for as long as three successive months. It has not been continued longer, and consequently we have no data as to whether the endometrial hyperplasia and profuse bleeding reported by Sturgis and Meigs might have resulted from longer use.

From these experiments and those of Sturgis and co-workers, it is apparent that prolonged or continuous treatment with estrogen will prevent ovulation and eliminate the luteal phase from the cycle.

LATE TREATMENT

It has been reported by Nelson and associates (7, 8) that estrogen maintained functional corpora lutea in the rat and Heckel and Allen (9) made similar observations in the rabbit. Westman (5) administered estrogen to

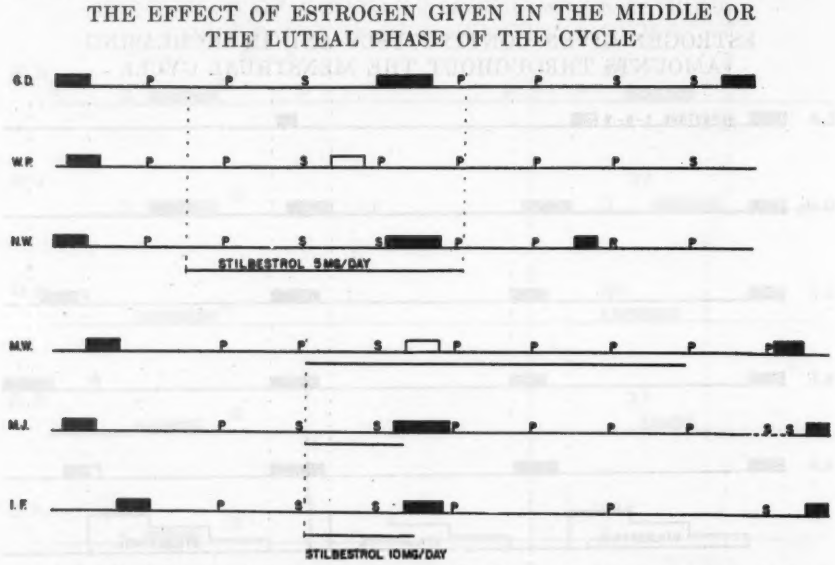

FIG. 4. Graphic representation of the menstrual intervals and endometrial findings in patients given stilbestrol through the luteal phase of the cycle. The horizontal lines represent the duration of treatment. The letters denote the condition of the endometrium, P for proliferative and S for secretory. The two open bars represent probable menstrual periods in patients who could not give reliable menstrual dates; the change in the endometrium indicated an unrecorded menstruation.

two women and then, on the basis of one premenstrual biopsy and one biopsy taken nine weeks after the previous period (in a patient with irregular cycles), presumed he had maintained luteal function in a manner similar to that demonstrated in the experimental animal.

Three women were given 10 mg. of stilbestrol daily starting in the postovulatory (luteal) phase (Fig. 4). There was no augmentation or prolongation of the luteal phase in these patients. One of the subjects (M. W.) was a mental patient and unable to give a menstrual history. The change from secretory to proliferative pattern in the endometrium indicated that the

patient had bled even though this fact was not recorded. It is interesting in her case that prolonging the estrogen treatment for an additional three weeks resulted in an anovulatory menstruation in the subsequent cycle; whereas, in the other two women the estrogen therapy was stopped with the onset of bleeding and the subsequent cycle was ovulatory.

In order to determine if earlier or more prolonged administration of estrogen would be effective, three women were given 5 mg. of stilbestrol daily starting about the tenth day of the cycle (Fig. 4). There was no increase in the length of the cycle and progestational changes in the endometrium were normal in degree and duration. These observations offer no evidence that the human corpus luteum is affected in any way by estrogen. On the basis of these observations it remains to be demonstrated whether estrogen will maintain functional corpora lutea in women as has been demonstrated in rats and rabbits.

DISCUSSION

From the foregoing observations, it is evident that a single dose of estrogen given early in the cycle may delay ovulation for a period of seven to ten days. When the estrogen is given over a period of time (fourteen to thirty days) ovulation is completely suppressed. However, if estrogen treatment is started after the follicle has matured or ovulation has occurred, it has no effect on the functional span of the corpus luteum. Thus the effect of estrogen varies with the dosage, the timing and the duration of treatment.

These experiments afford no evidence as to the mechanism whereby the estrogen exerts its effect. It is not possible to determine whether the estrogen may have some direct effect on the ovary or whether its effect is through the stimulation or suppression of gonadotropic activity of the anterior pituitary. Sturgis and Meigs (2) suggested that the estrogen "repressed" pituitary function. There is also the possibility that a single dose of estrogen early in the cycle can release hormone from the anterior pituitary before the Graafian follicle is mature enough to respond. This discharge of gonadotropin following estrogen was suggested by D'Amour (10). Experiments testing this hypothesis are reported elsewhere (11). Premature discharge of gonadotropin and a latent period for recovery of normal pituitary potency could account for the prolonged cycles when estrogen is given soon after a menstrual period. Continuous or prolonged estrogen treatment would delay restoration of pituitary hormone content so that anovulatory cycles would seem a logical result.

It has been shown experimentally that progesterone protects the pituitary from depleting its hormone content under the influence of estrogen. The lack of any effect of estrogen during the luteal phase may be due

to the presence of progesterone. The validity of this argument in human physiology should be tested by giving progesterone with the single dose of estrogen early in the cycle to determine whether the progesterone would negate the estrogen effect.

SUMMARY

The effect of estrogen on the human ovarian cycle varies with the mode of administration.

1. A single large dose (10 to 20 mg. of stilbestrol orally) given early in the cycle will delay ovulation for about ten days and increase the length of a cycle similarly. This schedule delays but does not inhibit or alter the normal sequence of ovulation and corpus luteum formation.

2. Estrogen (stilbestrol) given continuously for thirty days in daily doses increasing from 1 mg. to 3 mg. will completely inhibit ovulation. This can be repeated for at least three successive cycles without producing complications.

3. Estrogen (stilbestrol) started late in the cycle has no effect on the appearance, or on the duration of the luteal phase of the cycle. Stilbestrol given orally in doses of 10 mg. daily has not maintained functional corpora lutea in women.

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EFFECT OF LOW DOSAGE ROENTGEN RADIATION ON THE GONADOTROPIC FUNCTION OF THE HYPOPHYSIS OF THE MATURE AND IMMATURE FEMALE ALBINO RAT

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I. INTRODUCTION

IN VIEW of the wide interest and frequent use of low dosage roentgen therapy in the treatment of functional endocrine dyscrasias, and the many controversial theories concerning its effect on glandular function, it has seemed important to study the action of radiation on the normal pituitary of the experimental animal as manifested in its sexual system.

Although thirty-two years have elapsed since van de Velde (1) first used low dosage roentgen therapy as a therapeutic agent in the treatment of functional menstrual disorders and sterility, its mode of action on the endocrine glands is still very poorly understood. Many theories have been proposed to explain the good results reported clinically in hypofunctional endocrine conditions. All these theories can be divided into two main groups: 1) stimulation; 2) selective destructive action. Schwarz (2) believes a direct stimulation of glandular function is produced by small doses of x-rays, through an alteration of biochemical factors such as an increase of reversible cell permeability, or a rearrangement of electrons, atoms, and molecules, to increase the degree of cellular activity without the production of detectable changes in cellular structure. He also feels that an indirect stimulation may be mediated through the production of an active hyperemia in the irradiated gland, causing an increased blood supply, and resulting in an increased metabolic activity of the irradiated cells, Mazer and his co-workers (3, 4, 5, 6), Kaplan (7, 8) and several others (9, 10) refer to this theory of stimulation as the probable explanation of the 45 to 70 per cent clinical cures of various menstrual dysfunctions noted in their series of cases following radiation of the pituitary, the pituitary and ovaries or the ovaries. Thaler (11), Desjardins (12, 13), Tamis (14) and numerous other workers (15, 16, 17) refuse to accept this theory, and believe that x-rays, regardless of the smallness of the dose, always produce degenerative

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and destructive changes in the irradiated cells. They prefer to explain this apparent stimulation on the basis of a release of some check on the normal cyclic activity of the ovary, such as the destruction of a large Graafian follicle which is preventing the growth and maturation of other primordial follicles, or the destruction of a persistent corpus luteum which is suppressing menstruation. This is based on the experimental work of Van Pee and Simon (18), who irradiated the ovaries of mature rabbits and dogs and demonstrated a selective sensitivity of the various structures in the ovary to x-rays. The large maturing follicle was found to be the most sensitive and the corpus luteum the least sensitive, due in all likelihood to the less mature and more actively growing granulosa cells of the follicle.

Cystic ovaries have long been known to cause delay in menstruation Robinson (19) and Stein and his co-workers (20, 21, 22) have reported several cases of bilateral cystic ovaries associated with amenorrhea and sterility which were cured by surgical removal of the cystic portions of the ovary.

Good results have been reported by Steinhardt (23), Werner (24, 25) and others (26) following radiation of the pituitary alone in cases of amenorrhea and dysmenorrhea. Proponents of the theory that x-rays always have a destructive or inhibitory effect on cellular activity argue that the menstrual disorder in such cases probably was due to a hypersecretion of follicular stimulating hormone by the anterior lobe cells of the hypophysis, and that the reported cures resulted from an inhibition of these hyperfunctioning cells. Zondek's (27) experimental work has shown that amenorrhea and uterine bleeding are due in many instances to hypersecretion of the anterior lobe of the pituitary. If this condition persists for a long time histological evidence appears in the ovaries in the form of multiple cysts.

Newell and Pettit (28) reported a series of patients who were treated for various functional gynecological disorders. The blood of thirteen patients who received radiation of the pituitary was assayed for anterior pituitary sex hormone both before and after x-ray treatment. Ten patients showed an excess of anterior pituitary hormone in their blood before treatment. This excess hormone is reported to have disappeared after x-ray treatment in seven but persisted in the other three patients. In three patients the anterior pituitary hormone level in the blood was considered to be normal before treatment and did not change following pituitary radiation. Though the number of patients in which such studies were done is very small, they felt that there was a definite indication that this hormonal function of the pituitary gland was influenced (diminished) by even small doses of x-rays.

Thus the theories of the mode of action of small doses of x-rays on the glandular function are numerous, widely divergent, and contradictory. It

was decided therefore to study the effect of low dosage radiation on the normal hypophysis of the albino rat: 1) in order to determine whether there was any evidence of an alteration in glandular function, and 2) if any effect was demonstrated, by what mechanism it was produced.

II. REVIEW OF EXPERIMENTAL LITERATURE

The first studies of the effects of radiation of the pituitary in animals were made by Brunner (29) in 1920. He irradiated the entire head of young cats and dogs and reported some histological changes in the pituitary following fairly large doses of x-rays as well as slight histological changes in the nervous tissue and some suppression of growth. He stated that the pituitary was only slightly sensitive to x-rays. Fraenkel and Geller (30) in 1921 observed weight loss, growth retardation, and hypoplasia genitalis following radiation of the hypophysis of young female rabbits. In 1922, Rahm (31) irradiated the hypophyseal regions of young rabbits and observed a stimulation of growth with small doses and a cessation of growth with large doses. Podljaschuk (32, 33) in 1927 and 1928, using young and mature rabbits and dogs, and Mahnert, (34) using infantile mice, observed an inhibition of body growth and underdevelopment of sex organs. Epifanio and Cola (35) found that a 25 per cent erythema dose directed to the hypophysis of young rabbits accelerated growth, but that an erythema dose caused suppression of growth. With the erythema dose there were demonstrable histological changes in the pituitary, the acidophiles being the most affected. Selle, Westra, and Johnson (36) using doses as high as 3000 r directed to the pituitary of depancreatized dogs were unable to ameliorate their diabetes. Histologically, the hypophysis showed congestion and degeneration; degenerative changes were found in the nervous tissue of the brain.

Lacassagne (37) introduced radon and radium needles into the hypophyseal glands of rabbits and found partial to complete destruction of the gland after long periods of exposure. Associated with the pituitary necrosis he observed loss of sex drive and gonadal atrophy. He found that if one-third of the gland remained, this was sufficient to produce a normal function of the ovary, and concluded that the pituitary was very radioresistant.

Fehr (38) studied the effects of both massive and fractionated doses of roentgen radiation over the pituitary regions of mature rabbits. He administered doses up to 10,160 r to adult rabbits in amounts of 600 r over a period of two to three months, but did not demonstrate any physiological effect, and no histological changes were found in the pituitaries when the animals were sacrificed four to six weeks later. With single massive doses of 2860 r there was a demonstrable weight loss, diminution of sex drive, and the ovaries and uterus were juvenile in appearance. He calculated the tissue

dose to the pituitary to be about 80 per cent of the skin dose and concluded that the rabbit pituitary was very radioresistant and could not be influenced by ordinary doses of x-rays.

The results observed by many of these early workers were varied and often contradictory, but the majority of the studies seem to indicate that the hypophysis of these various laboratory animals was relatively radioresistant. However, it must be remembered that much of this early work was done with x-ray equipment that was inefficient by modern standards, and since there was no accepted dose standardization, there was often no real basis for comparison of the results of one worker with those of another.

Lawrence, Nelson, and Wilson (39) studied the effects of radiation of the hypophysis in immature albino rats using factors of 180 kv., 25 ma., filter 0.5 mm. Cu and 1 mm. Al, 50 cm. distance, and a 1 cm. circular portal. With doses of 513 (air) r repeated at three or four sittings about five days apart, there was only a slight inhibition of gain in weight, but when two treatments of 1040 (air) r were given at a similar interval, there was a definite suppression of growth and ovarian activity. The pituitaries, ovaries, thyroids, and adrenals of irradiated animals sacrificed eighteen to twenty-two days after x-ray therapy were definitely decreased in size. Histologically, there was a scarcity of acidophile cells in the hypophysis and the basophile cells, though normal in number, showed some distinct clumping of the granules and frequent pyknotic nuclei. This damage, however, was not permanent as there was a gradual return of the hypophyseal structure and function to normal in animals sacrificed two to three months after treatment. They conclude that although the pituitary glands of young albino rats are relatively radioresistant, nevertheless they can be influenced by x-rays.

Because of the numerous references to cures of sterility in women by means of "stimulating" doses of x-rays, Hartman and Smith (40) in 1938 made a study of the effects of small single doses of from 60 to 400 (air) r on 13 sterile female rhesus monkeys, using factors of 200 kv., 20 ma., filter 0.5 mm. Cu and 1 mm. Al, 50 cm. distance, 4 by 5 cm. field. Although their findings were somewhat indefinite, they concluded that x-rays in the doses used had no effect on the pituitary of these animals. Kotz, Edward, and Parker (41) irradiated the hypophyses of 20 adult female rats with single doses of 200 to 500 r using factors of 200 kv., 25 ma., filter 0.5 mm. Cu and 1 mm. Al, 50 cm. distance. The animals were sacrificed from three to seven weeks after roentgen treatment and histological studies were made of the pituitaries, ovaries, thyroids, and adrenal glands. No degenerative changes were noted in these endocrine glands. They concluded that radiation of the pituitary in the doses used had no harmful effects.

Denniston (42) in 1942 irradiated the pituitaries of adult and immature

ground squirrels and albino rats. He used factors of 200 kv., 4 ma., filter 0.5 mm. Cu and 1 mm. Al, 60 cm. distance. Doses varying from 7 to 140 r were used in immature ground squirrels, and at autopsy three to twelve weeks after treatment, he found the pituitary and sexual organs of the treated animals in no way different from those of the controls. Most of the animals were treated with doses ranging from 500 to 4000 r. He reached the following conclusions: 1) The pituitary is extremely resistant to x-rays. 2) No good evidence of a "stimulating dose" has been found from these studies. 3) A differential susceptibility of the anterior lobe cells is demonstrated; the alpha cells lose their characteristic granules, the cytoplasm shrinks, and there is an increase in size of their nucleoli; the beta cells and chromophobes are apparently unaffected within the dosage of 500 to 4000 (air) r used in the ground squirrel. 4) The growth rate of young rats is reduced 50 per cent following radiation of the pituitary with 1000 (air) r or more.

Hodes and Israel (43) treated adult female rabbits with doses of 50 to 80 (air) r to both the ovaries and pituitary using factors of 200 kv., 15 ma., half-value layer 1 mm. Cu, distance 50 cm., and could demonstrate no evidence of ovulatory stimulation following radiation.

While our study was in progress, Baidins, Claesson and Westman (44) were also studying the effects of radiation of the pituitary in infantile rats using factors of 105 kv., 10 ma., filter 1 mm. Al and 165 kv., 7 ma., with 0.5 mm. Cu filtration. Their results were reported in 1946. With single treatments of 2 to 45 r, they noted an increase in the size and weight of the pituitaries of the treated animals sacrificed from eleven to fourteen weeks after treatment. The only histological change was a slight hyperemia of the pituitaries. Similar observations were made after single doses of 50 to 500 r but in these rats the hyperemia was more marked and there was a greater increase in the size and weight of the pituitaries. The ovaries of the treated rats were in no way different from the controls. Even though the increase in size and weight of the pituitary seemed to indicate a stimulating effect of radiation on the pituitary, they were unable to demonstrate any evidence of an increase in function of either the pituitary or ovarian cells. They therefore concluded that it was impossible to influence the pituitary gland of the white female infantile rat by small doses of x-rays.

Though a review of these recent experimental studies with low dosage radiation of the pituitary (doses of 400 (air) r or less) has shown no histological effects on the sexual system of animals, the immediate and latent physiological effects were seldom carefully observed. In view of the importance of this highly controversial problem, we thought that further investigation was indicated using methods designed chiefly to study and evaluate any physiological effect that might be produced.

III. MATERIALS AND METHODS

Ninety *sexually mature* female Wistar albino rats were used in the first part of this study. The Wistar albino rat was chosen as the test animal since it has a short oestrus cycle and experimental methods have been established for determining the various phases of its oestrus cycle. The length of its cycle is 4 or 5 days and there are five different stages; each is characterized by periodic changes in the epithelium of the uterus and vagina. The various phases of the cycle can also be determined from the rat's running activity. There is a marked rise in the activity of the rat on the first day of the cycle, day of heat, and period of ovulation. The activity then decreases and remains low throughout the next two days and then begins to rise slowly on the fourth day with a marked peak again on the first day of the next cycle.

Farris (45) has studied a large number of Wistar female albino rats over long periods of time and has found the activity records more satisfactory than vaginal smears for determination of the different stages of the cycle.

The signs of heat in the rat are as follows:

1. Ear quivering (EQ) elicited by stroking gently the head or back.
2. Lordosis or copulation response (CR) produced by digital stimulation of the pelvic region.

Whenever the symbol "EQCR"[1] is used it indicates the presence of the combined response to "EQ" and "CR," as referred to above.

When in heat the rat is nervous, apprehensive, darting about, and on being touched, braces herself. This period of heat usually lasts from eight to twelve hours, and it is only during this time that she will accept the male.

The rats were kept in a special colony room at The Wistar Institute. The colony room was darkened completely from 8 a.m. to 8 p.m. Four 100 watt lights were turned on automatically at 8 p.m. and remained on until 8 a.m. By this method the rats display more regular oestrus. On the first day of the cycle heat occurs about 9 a.m., thus bringing it within our working day.

Activity determinations were made by keeping the rats in cages equipped with turntables connected to electrical counters which recorded each revolution of the turntables. Activity records were kept on all the rats, and they were checked for signs of heat on the day of oestrus.

Roentgen treatments were given at 7.30 a.m. on the second day of the cycle in all of the irradiated rats, except in five which were treated at 7.30 a.m. on the third day. No rats were treated unless signs of heat had been elicited on the preceding day of oestrus and were absent on the morning

[1] Abbreviation used in tables in text.

of the second day. After treatment they were returned immediately to their cages and checked at half-hour intervals for signs of heat, for a period of three hours following radiation of the pituitary. For each two treated rats, one control was taken out of its cage and handled similarly to the irradiated rats, but received no radiation.

Six treated rats and seven of the day-two control animals were sacrificed and studies made of the pituitaries, ovaries, and uteri. These organs were then compared with the organs of six untreated rats sacrificed on day-one, the normal day of oestrus.

Twenty-two *immature* female Wistar albino rats twenty-eight days in age were used in the second part of this study. Vaginal opening was taken as the sign of sexual maturity. Eight of these rats were treated and followed daily until vaginal opening occurred. Two others were used as controls. An additional eight were treated, and together with four controls, were sacrificed when forty days of age and pituitary, ovarian, and uterine weight determinations were made at this time.

The following *method of treatment* was used throughout the experiment: The rats were treated with an intermediate therapy unit using factors of 135 kb., 8 ma., inherent filtration 1 mm. Al with 2 mm. Al added giving a half-value layer of 4 mm. Al. A circular cone 1.8 cm. in diameter was used with a distance of 24 cm. from the target to skin surface. The roentgen output at the end of the cone was 90 (air) r per minute. The pituitary gland of the rat is located at an approximate depth of 1 cm. from the skin surface. Depth dose measurements were determined at 1 cm. using a fiberwood phantom and then expressed in terms of the air roentgens as measured at the skin surface. This gave a depth dose measurement at 1 cm. equal to 76 per cent of the air roentgens delivered at the skin surface. The doses are given in terms of air roentgens throughout this report and can be reduced to tissue roentgens by multiplying by the above percentage factor.

The rat was immobilized by wrapping it tightly in a cotton cloth and then molding a lead cage about the body so that only its head was exposed. A lead collar was slipped about the neck. The treatment cone was then brought down on the head and centered over the pituitary region. Treatment sometimes had to be interrupted to re-position the rat's head in case of movement, but due to the relatively short treatment times this difficulty was minimized.

IV. RESULTS

(A) EFFECTS OF RADIATION OF THE PITUITARY ON THE SEXUAL ACTIVITY OF THE MATURE FEMALE RAT

1. *Single Dose.* Fifty-nine female rats varying in age from seventy-seven to two hundred and seventy days were used. All had regular four or five day

TABLE 1. RESULTS FOLLOWING SINGLE DOSES OF ROENTGEN RADIATION DIRECTED TO THE PITUITARY OF THIRTY-NINE SEXUALLY MATURE ALBINO FEMALE RATS

RAT			DOSAGE	EFFECTS OF TREATMENT	
Identification number	Age in days	Day of oestrus cycle	In (air) roentgens	EQCR* response	Mating
77′	240	2	10	none	no
68	245	2	10	none	no
62	270	2	20	none	no
67′	83	2	25	none	no
302	142	2	25	none	no
60′	233	2	50	none	no
219	128	2	50	none	no
208	136	2	50	EQCR	mating
217	93	2	50	EQCR	mating
106	183	2	50	EQCR	no
202	138	2	100	EQCR	mating
220′	189	2	100	EQCR	mating
303	133	2	100	EQCR	mating
73	212	2	100	none	no
70′	190	2	100	none	no
74	237	2	100	none	no
203	133	2	150	EQCR	mating
76[2]	106	2	150	none	no
75′	206	2	150	none	no
66	141	2	200	none	no
61	245	2	200	none	no
102′	77	2	200	none	no
103′	77	2	200	none	no
105	182	2	200	none	no
206	144	2	200	none	no
69′	83	2	200	none	no
301	131	2	200	none	no
97′	106	2	200	EQCR	no
78	217	2	200	EQCR	mating
65	240	2	200	EQCR	mating
90	234	2	200	EQCR	no
67′	245	2	200	EQCR	no
95′	126	2	200	EQCR	no
69	343	2	200	EQCR	no
72′	106	3	150	none	no
64	213	3	100	none	no
79	235	3	200	none	no
71[2]	149	3	200	none	no
98	247	3	200	none	no

* Symbol EQCR used in this table indicates ear quivering and copulation response.

cycles as shown by their running activity. Single doses of radiation to the pituitary varying in amount from 10 to 200 r were given to thirty-nine of these rats as shown in Table 1; twenty were used as controls.

Thirty-four of these thirty-nine rats were treated on the morning of the second day of the cycle. None of five rats of this group receiving less than 50 r showed any evident response to the radiation treatment. However, three of five rats given 50 r; three of six rats given 100 r; one of three rats

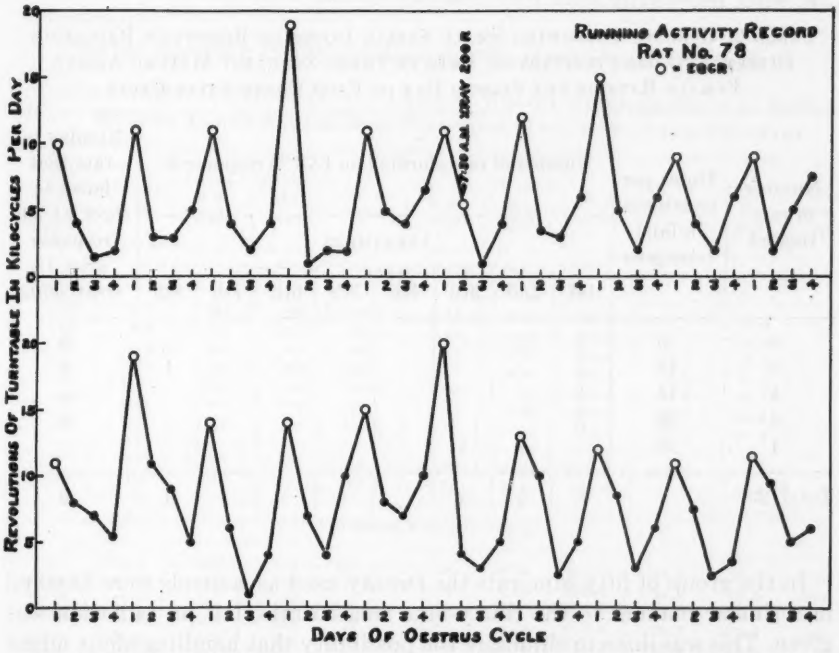

FIG. 1. Typical activity record of one rat (No. 78) in whom an EQCR response was noted on day-two of the oestrus cycle following a single treatment of 200 roentgens to the pituitary.

given 150 r; and seven of fifteen rats given 200 r showed signs of heat from one to two hours after treatment, which persisted for four to twelve hours. These findings were similar to those observed on the normal day of heat, which day is day-one of the cycle, differing only in that the running activity did not increase but tended to drop off, as in the untreated rat. Thus, a reappearance of heat signs was observed in fourteen (41.2 per cent) of these thirty-four rats following radiation, while twenty appeared to be unaffected by the treatment. The activity records of these rats were followed for 20 to 30 cycles after treatment and no change was noted in the periodi-

city of their cycles. A typical activity record of one of these rats in whom an EQCR response was noted following a treatment of 200 r is shown in Figure 1. All of the fourteen rats in whom an EQCR response was obtained on day-two following pituitary radiation were checked for mating and eight or 57.3 per cent accepted the male and two pregnancies resulted.

The remaining five of the thirty-nine rats were treated on the morning of day-three of the cycle with doses varying from 100 to 200 r, but none of them appeared to be affected in any way by the treatment.

TABLE 2. RESULTS FOLLOWING SMALL SERIAL DOSES OF ROENTGEN RADIATION DIRECTED TO THE PITUITARY OF TWENTY-THREE SEXUALLY MATURE ALBINO FEMALE RATS ON THE SECOND DAY OF EACH CONSECUTIVE CYCLE

Number of rats treated	Doses per treatment in (air) roentgens	Number of rats showing an EQCR response to TREATMENT								Number of rats that failed to show ECQR response after 15 treatments
		1st	2nd	3rd	4th	5th	6th	7th	8th	
9	5	3	2	1						3
6	10	1							1	4
1	15	1								—
6	20	3				1				2
1	25	—	—	1						—
Total 23		8	2	2		1			1	9

In the group of fifty-nine rats the twenty used as controls were handled in a similar manner to the thirty-nine treated rats, but no radiation was given. This was done to eliminate the possibility that handling alone might account for the EQCR response noted following treatment. None of the twenty controls showed any signs of heat on day-two or change in the regularity of their cycles as a result of this handling.

2. *Serial Doses.* A group of thirty-one sexually mature female rats was used to study the effect of serial treatments to the pituitary. Twenty-three of these received radiation and eight were used as controls. Radiation was given in doses from 5 to 25 r on the second day of each cycle. These same doses were repeated for as many as 10 to 15 consecutive cycles to determine whether there was any prolonged effect. The largest total treatment received by any one rat was 300 r. A summary of the EQCR response obtained by this method of serial radiation of the pituitary is shown in Table 2.

Three of the nine rats receiving 5 r responded to the initial treatment, two responded to the second treatment, one to the third treatment, and three were unaffected after fifteen treatments.

One of six rats given 10 r responded to the first treatment, one responded after eight treatments, and four were unaffected after fifteen treatments.

One rat receiving 15 r responded to this initial dosage.

Three of six rats given 20 r responded to a single treatment, one responded after five such treatments, and two were unaffected after fifteen treatments.

One rat receiving 25 r responded after the third of such treatments.

TABLE 3. PITUITARY, OVARIAN, AND UTERINE WEIGHT DETERMINATIONS IN SEXUALLY MATURE ALBINO FEMALE RATS FOLLOWING RADIATION OF THE PITUITARY

Rat identification number	Pit. wt. in mg.	Pit. wt./B.W. $\times 10^{-4}$	Pit. wt./B.L. $\times 10^{-4}$	Ov. wt. in mg.	Ov. wt./B.W. $\times 10^{-4}$	Ov. wt./B.L. $\times 10^{-4}$	Ut. wt. in mg.	Ut. wt./B.W. $\times 10^{-4}$	Ut. wt./B.L. $\times 10^{-4}$	Treatment in (air)
CONTROL RATS (DAY-TWO)										
104	11.7	.540	.585	108.1	4.66	5.24	505.6	21.8	24.4	
68	9.6	.424	.480	75.0	3.32	3.75	317.6	13.8	15.6	
73	10.4	.507	.525	83.1	4.05	4.20	359.3	17.5	18.1	
102	12.3	.545	.600	105.7	4.67	5.15	632.8	28.0	30.9	
75	12.0	.629	.609	110.5	5.79	5.61	604.5	31.6	30.7	
78	14.1	.572	.678	99.7	4.03	4.80	532.7	21.5	25.6	
77	12.5	.553	.598	87.2	3.86	4.18	555.9	22.9	26.2	
Total:	82.6	3.770	4.075	669.3	30.38	32.93	3508.4	157.1	171.5	
Average:	11.8	.538	.582	95.6	4.34	4.70	501.2	22.4	24.5	
TREATED RATS (DAY-TWO)										
215	16.5	.665	.774	106.2	4.28	4.98	607.5	24.5	28.5	200r
220	15.6	.596	.712	111.2	4.35	5.18	613.9	24.1	28.6	100r
218	13.0	.552	.617	120.6	5.00	5.65	529.8	21.9	24.8	10r
205	14.7	.596	.688	70.7	3.02	3.39	682.9	29.2	32.6	10r
214	15.2	.630	.730	79.6	3.06	3.76	744.0	28.2	35.1	5r
204	14.8	.641	.711	104.7	4.52	5.04	511.3	22.7	24.6	10r
Total:	89.8	3.680	4.232	593.0	24.23	28.00	3689.4	150.6	174.2	
Average:	14.9	.613	.705	98.8	4.04	4.66	614.9	25.1	29.0	
UNTREATED RATS IN OESTRUS (DAY-ONE)										
71	13.4	.556	.641	101.9	4.61	4.94	828.4	34.4	39.6	
211	14.1	.576	.659	112.3	4.54	5.20	1143.1	46.6	53.3	
305	12.5	.558	.601	107.6	4.81	5.18	1112.3	49.6	53.4	
200	12.5	.546	.616	101.4	4.45	5.03	1178.7	51.5	58.1	
95	14.3	.622	.697	103.9	4.51	5.06	1084.5	47.1	52.6	
74	13.6	.670	.687	82.9	4.08	4.19	971.1	47.4	49.0	
Total:	80.4	3.528	3.901	610.0	27.00	29.60	6318.1	276.6	306.0	
Average:	13.4	.588	.650	101.6	4.50	4.93	1053.0	46.1	51.0	

Pit. Wt. = Pituitary weight.
Ov. Wt. = Ovarian weight.
Ut. Wt. = Uterine weight.
B.L. = Body length.
B.W. = Body weight.

Seven of the fourteen rats in whom an EQCR response was observed following serial treatments accepted the male and two pregnancies and one pseudopregnancy resulted.

The EQCR response in these rats following serial treatments was similar to that noted on the normal day of heat except that their running activity was not stimulated. The periodicity of their cycles was unchanged. Most of these rats after showing a response to the radiation of the pituitary continued to show a similar response to several subsequent treatments.

TABLE 4. AVERAGE DIFFERENCE IN ORGAN WEIGHTS BETWEEN UNTREATED FEMALE RATS IN NORMAL OESTRUS AND UNTREATED DAY-TWO CONTROLS

Average	Pit. wt. in mg.	Pit. wt./ B.W. $\times 10^{-4}$	Pit. wt./ B.L. $\times 10^{-4}$	Ov. wt. in mg.	Ov. wt./ B.W. $\times 10^{-4}$	Ov. wt./ B.L. $\times 10^{-4}$	Ut. wt. in mg.	Ut. wt./ B.W. $\times 10^{-4}$	Ut. wt./ B.L. $\times 10^{-4}$
In rats in normal oestrus	13.4	.588	.650	101.6	4.50	4.93	1053.0	46.10	51.00
In day-two control rats	11.8	.538	.582	95.6	4.34	4.70	501.2	22.40	24.50
Difference in weight +	1.6	.05	.068	6.0	0.16	0.23	551.8	23.70	26.55
Difference in per cent +	13.5	9.20	11.60	6.2	3.60	4.90	110.0	105.00	108.00

+ =increase.

The eight controls of this group of thirty-one rats received no radiation and they showed no signs of heat on day-two as the result of handling.

3. *Gross Anatomical Studies.* Six of the rats who showed an EQCR response following radiation treatment on day-two were sacrificed six hours later together with seven day-two controls. The pituitary, ovaries, and uterus were removed and weight determinations made. These were then compared with similar studies made on six untreated rats sacrificed on day-one, the normal day of oestrus. The ratio of these organ weights to that of the whole body weight and body length of the rat were calculated for all three of these groups, to make allowances for differences in the weight and size of the animals. The weight determinations and the average for the three groups are shown in Table 3.

The average percentage differences in the organ weights of the untreated rats sacrificed on the day of oestrus as compared to the untreated control rats killed on day-two is shown in Table 4. The average pituitary weight was 13.5 per cent greater in the rats in normal oestrus, but this difference is reduced to 9.2 per cent due to the average greater body weight in this group. There is no significant difference in ovarian weights when the averages for the two groups are compared. The average uterine weight of

the rats in normal oestrus however, was 110 per cent above that in the two-day controls.

The average percentage differences in the organ weights of the treated rats as compared to the untreated control rats sacrificed on day-two are shown in Table 5. The average pituitary weight was 26.2 per cent greater in the treated rats when compared to that of the controls but this difference is reduced to 13.9 per cent when body weight variations are considered. The average ovarian weight was essentially the same in the two groups.

TABLE 5. THE AVERAGE DIFFERENCE IN ORGAN WEIGHTS BETWEEN FEMALE RATS TREATED ON DAY-TWO AND THE DAY-TWO CONTROLS

Average	Pit. wt. in mg.	Pit. wt./ B.W. $\times 10^{-4}$	Pit. wt./ B.L. $\times 10^{-4}$	Ov. wt. in mg.	Ov. wt./ B.W. $\times 10^{-4}$	Ov. wt./ B.L. $\times 10^{-4}$	Ut. wt. in mg.	Ut. wt./ B.W. $\times 10^{-4}$	Ut. wt./ B.L. $\times 10^{-4}$
In day-two treated rats	14.9	.613	.705	98.8	4.04	4.66	614.9	25.10	29.00
In day-two control rats	11.8	.538	.582	95.6	4.34	4.70	501.2	22.40	24.50
Difference in weight +	3.1	.075	.123	3.2	—	—	113.7	2.70	4.50
Difference in per cent +	26.2	13.90	21.10	3.3	—	—	22.6	12.00	18.40

+ =increase.

The average uterine weight was 22.6 per cent greater in the treated rats but this difference is reduced to 12 per cent on considering body weight and body length variations.

(B) EFFECTS OF RADIATION OF THE PITUITARY ON THE DEVELOPMENT OF THE SEXUAL SYSTEM OF THE IMMATURE FEMALE RAT

1. *Single and Serial Doses.* Twenty-two immature female Wistar albino rats twenty-eight days in age were used in this part of the study.

Of the first ten rats studied, eight were treated by radiation of the pituitary in small doses varying from 5 to 50 r, and two were used as controls. A pair of rats was given doses of 5 r, one receiving only a single treatment whereas the other received five treatments on successive days. Similarly the other three pairs were treated with single and with five daily doses of 10, 20, and 50 roentgens, respectively, as shown in Table 6. The maximum dose received by any one rat was only 250 r. These rats were then followed daily until vaginal opening occurred. One died of unknown cause before the study was completed. Vaginal opening occurred at ages ranging from fifty-three to sixty-five days in both the controls and treated rats. This is

within the normal limits for the onset of sexual maturity as manifested by vaginal opening, according to the work of Long and Evans (46).

Eight of the remaining twelve immature rats were then similarly treated with single and five daily treatments of 10, 20, 50, and 100 roentegns and the other four were used as controls. These animals were sacrificed twelve days after radiation of the pituitary and pituitary, ovarian, and uterine weight determinations were made as was done with the sexually-mature rats. There was no significant organ weight difference between these

TABLE 6. RESULTS OF RADIATION OF THE PITUITARY ON THE ONSET OF PUBERTY IN 28-DAY-OLD FEMALE RATS AS DETERMINED BY THE TIME OF VAGINAL OPENING

Rat identification number	Dose administered per treatment in (air) roentgens	Total number of successive daily treatments	Age at vaginal opening in days
0	5	1	53
1	5	5	63
2	10	1	53
3	10	5	58
4	20	1	59
5	20	5	died
6	50	1	65
7	50	5	56
8	control	1 handling only	59
9	control	5 handling only	57

eight treated rats and their four controls. Grossly the appearance of these treated organs was in no way different from the control untreated organs.

V. DISCUSSION

In a study of the effects of various pituitary and ovarian hormone preparations on the rhythm of the oestrus cycle and running activity of the rat, Farris (47) was able to show that the appearance of the clinical signs of heat was dependent upon the secretion of follicle-stimulating hormone by the pituitary and the resultant stimulation of estrogen production in the ovary. The increased running, however, was largely due to estrogen. Urine extracts high in their luteinizing effect when injected into the normal rat on the day of peak activity caused a sudden marked drop in its activity and disappearance of heat signs. Similarly injections of progesterone caused a decrease in activity but this was not as marked as that following injections of pituitary luteinizing hormone. Thus he was able to correlate the running activity of the rat with the various hormone preparations believed to be involved in the production of the oestrus cycle.

Signs of heat, mating, and an increase in running are normally observed during the period of oestrus, on the first day of the oestrus cycle. This period of oestrus lasts from eight to twelve hours. Farris, (45) in a previous study of a large number of rats, has observed only occasionally these physiological signs of heat on the second day of the cycle or the metoestrus period.

None of our twenty-eight controls in the sexually mature group handled similarly to the irradiated rats in this group showed any signs of heat or change in the regularity of their cycles as the result of handling.

Thus, the reappearance of heat signs on day-two of the oestrus cycle, from one to two hours after single or multiple doses of radiation of the pituitary in twenty-eight (49.1 per cent) of fifty-seven sexually mature female albino rats, seems to indicate that x-rays can produce a transient effect on pituitary function. Fifteen (53.5 per cent) of these twenty-eight rats accepted the male, resulting in four pregnancies and one pseudopregnancy. Is this transitory effect due to a sudden release of follicle-stimulating hormone by the pituitary as a result of an increased vascularity or increased cell permeability secondary to cellular injury following radiation? That such initial changes can be observed in tissues immediately following roentgen therapy is well known. Ewing (48) states that this initial hyperemia seems to be merely a somewhat peculiar inflammatory process with vasodilatation, exudation of serum, leukocytes, and red cell infiltration. An increased permeability of the cell membrane may result from cellular damage.

Gross studies showed the pituitaries of six of our irradiated sexually mature rats to be more erythematous in color than those of the day-two controls. In addition, the average weight of the pituitary glands of the treated rats was at least 13.9 per cent greater than that of the controls. This increase in weight of the pituitary gland following radiation is similar but slightly greater than that found in rats in normal oestrus. An increase in uterine weight is believed to be due to estrogen secretion. Though the average uterine weight was at least 12 per cent greater in the treated rats when compared to that of the controls, suggesting some increased estrogen secretion by the ovary following radiation of the pituitary, this increase in weight was not nearly as marked as the 105 per cent noted in rats during normal oestrus. This possible increased estrogen secretion was apparently not sufficient to produce an increase in running.

No EQCR response was obtained in 50.9 per cent of the fifty-seven sexually mature day-two irradiated rats. This failure may have been due to the fact that in these rats metoestrus had already become sufficiently well-established so that even though additional follicle-stimulating hormone was released following radiation of the pituitary, it was not sufficient to

overcome the antagonistic action of the pituitary luteinizing hormone, or progestin, which is predominant during this phase of the cycle. This assumption is supported by the fact that no EQCR response was obtained in the five sexually mature rats irradiated on day-three.

Though these results show that radiation has a transient effect on the function of the pituitary gland of the adult rat, no prolonged effect could be demonstrated since the activity and rhythm of the cycle was not altered in rats followed for over 30 cycles after roentgen therapy.

Such a transient apparent stimulatory effect of radiation has previously been reported following low dosage radiation in growing plants and animals. Johnson (49), in a review of the literature regarding the influence of x-rays on the processes of germination and seedling growth, found that most workers who reported an increased germination or increased growth in vegetative parts following small doses of x-rays had based their conclusions on short time experiments or had used only a limited number of seeds. When their experiments were repeated with large numbers of seeds under well controlled conditions, and the rate of growth was followed until plant maturity, the results showed either no effect or actual inhibition of growth.

An excellent series of experiments was carried out by Arntzen and Krebs (50) in 1925 on germinating peas. They exposed peas in process of germination to graded doses of roentgen rays, and found that the plants receiving very small doses grew faster than the controls during the first twenty-four to forty-eight hours, but that their rate of growth diminished steadily thereafter and the final result was an actual inhibition.

Several workers have reported a stimulation of growth in embryos and young animals following radiation with small doses, but the majority have observed either no effect or a retardation of growth. Desjardins (12) in reviewing and discussing these conflicting results, states that reports of an accelerated growth rate in animals following low dosage radiation of the pituitary were based on observations carried out over a short period of time, and that had these workers observed their experimental animals over a longer period an actual retardation of growth would have been recorded. He cites the work of Gillman and Baetzer (51) who, on exposing the eggs of amblystoma for varying periods, observed during the first ten days acceleration of development, but later, retardation; and the final irradiated products were all abnormal. Further experiments on the development of chicks following exposure of the eggs to roentgen rays yielded the same result.

Thus, the overwhelming evidence in the experimental literature indicates that x-rays even in small doses are not capable of directly stimulating living cells, if by the term "stimulation" is meant a continued acceleration of cellular growth and metabolism. Any transient acceleration of cellular

growth or metabolism as observed in this study, and reported by numerous other workers, may therefore be regarded as secondary to an increased vascularity or altered cell permeability that is produced in the irradiated tissue by even very small doses of x-rays. Very small doses of x-rays thus appear to have an irritational effect on the irradiated cells without producing any microscopic evidence of injury. Desjardins believes that such an apparent stimulation is invariably followed by more or less pronounced functional organic deterioration. There was no evidence in our studies, however, of any harmful effects either organic or functional following radiation of the pituitary in the doses used in our investigations. Whether such a mild irritational effect produced by these small doses of x-rays is capable of "waking up" an abnormal or hypofunctioning gland as sometimes postulated in the medical literature, is open to some question.

Though the results of radiation of the pituitary in the sexually mature rat did not suggest any prolonged effect of x-rays on glandular function and the previous reports of a stimulative effect on growth following low dosage radiation of the pituitary had been largely proved erroneous, it was nevertheless felt that this study would not be complete without evaluating the effects of such radiation on the immature animal. This seemed important in view of the fact that the dosage used by early workers could not be accurately determined, and therefore an evaluation and comparison of their results was difficult.

Denniston's (42) studies on immature ground squirrels in 1942 had shown no effect of small doses of x-rays from 7 to 140 r on the development of their sexual systems. It is not known whether his findings subsequently have been confirmed by any other investigator. However, the experimental literature contains numerous studies showing that a premature onset of puberty can be produced in twenty-two day old rats by injecting gonadotropic hormonal preparations.

Smith, Engle and Tyndale (52, 53) found that injections of extracts of follicle-stimulating urine in premature rats twenty-two days in age was effective in causing enlargement of the ovary, increased development of the follicles, and opening of the vagina. Corey (54) produced ovulation in about one-third of rats twenty-one to twenty-eight days old with 2 to 16 rat units of pituitary gland extract. Cole (55) in studying the effects of mare gonadotropic hormone on twenty-one to twenty-six day old female rats was able to induce true sexual maturity as judged by the ability of these immature rats to produce living young.

If small doses of follicle-stimulating hormone were capable of producing sexual precocity in the immature rat, perhaps then, if small doses of radiation of the pituitary were able to stimulate cell growth and metabolism, a similar result might be observed? This, however, did not prove to be

the case since neither single nor serial doses of x-rays in the dosages used in this study had any effect on the development of the sexual system of sixteen immature twenty-eight day old female rats. This is presented as further evidence that x-rays cannot directly stimulate normal cellular growth.

VI. SUMMARY

Five sexually mature female Wistar albino rats were given single doses of roentgen radiation to the pituitary varying from 100 to 200 r, at 7.30 a.m. on the third day of their oestrus cycles and none appeared to be affected by treatment.

Fifty-seven sexually mature female Wistar albino rats were given single or multiple doses of roentgen radiation to the pituitary varying from 5 to 300 r, at 7.30 a.m. on the second day of their oestrus cycles.

Twenty-eight additional rats were used as controls.

Physiological signs of heat, ear quivering and copulation response (EQCR), were observed in twenty-eight (49.1 per cent) of the fifty-seven rats from one to two hours following treatment. These signs of heat lasted for six to eight hours. Fifteen of these twenty-eight rats accepted the male. Four pregnancies and one pseudopregnancy resulted. The running activity was recorded for 20 to 30 cycles after treatment. No change was noted in the running activity or periodicity of the oestrus cycle of the treated rats.

Gross anatomical studies and weight determinations were made of the pituitaries, ovaries, and uteri of six of the day-two treated rats who showed an EQCR, as well as of the seven day-two controls. The pituitary weights of the treated rats appeared to be significantly greater than those of the controls. The ovarian weights were similar in the two groups. The uterine weights of the treated rats were slightly greater than those of the controls, but this increase in uterine weight was not nearly as definite as that noted in rats during the normal oestrus.

Sixteen immature female Wistar albino rats were given either single or five successive daily roentgen treatments to the pituitary with doses varying from 5 to 100 r per treatment.

Six additional rats were used as controls.

Eight of the immature treated rats were followed until vaginal opening occurred at ages ranging from fifty-three to sixty-five days. This is within the normal limits of age for the onset of sexual maturity.

Eight additional immature treated rats and four controls were sacrificed twelve days after radiation of the pituitary, and pituitary, ovarian, and uterine weight determinations made. Grossly the appearance and weight of the organs from the treated immature rats were in no way different from their controls.

VII. CONCLUSIONS

As a result of these studies the following conclusions were reached:

1. Low dosage radiation of the pituitary can produce a transient effect on the normal pituitary function of the rat. Though the immediate physiological effect is in the nature of a stimulus, such an effect is only transient and not prolonged.

2. Radiation of the pituitary in small doses has no effect on the normal development of the sexual system of immature twenty-eight day old female rats.

3. There is no evidence that x-rays can stimulate living cells, if by stimulation is meant a continued acceleration of their normal growth or function.

4. Radiation of the pituitary in small doses has no evident harmful effect in the experimental rat.

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MYXEDEMA WITH DELAYED CLOSURE OF EPIPHYSES IN SEXUALLY MATURE WOMEN

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IT IS generally believed that closure of the epiphyses is caused by the sex hormones. In all insufficiencies of the gonads, whether primary, or secondary to insufficiency of the anterior lobe of the hypophysis, a delay in synostosis is observed. This anomaly of bone age, however, is also to be seen in thyroid deficiency. It is present not only in the myxedema of infants and adolescents but also in the myxedema of mature women who have menstruated regularly for a long time. Thus Mussio Fournier and Proto (1) have published the case of a myxedematous woman 32 years of age, untreated with desiccated thyroid, in whom menstruation had occurred with normal rhythm since the age of 22 years. In this patient some epiphyseal cartilages still existed.

Besides the case first mentioned we now present four cases of myxedematous women in whom similar conditions were observed.

CASE HISTORIES

No. 9156: P. S. was a woman 32 years of age. She was first seen by us because of a clinical picture of myxedema (Fig. 1). As an infant, teething, walking and speech followed their normal course. She was sensitive to cold from childhood and was habitually constipated. The menarche occurred at 20 years of age and was followed by regular menstruation. The symptom that brought her to our service was edema of the face and limbs which had existed for one year.

Physical examination: The patient was short, (height, 143.0 cms.) and had typically myxedematous facies, dry skin and scalp hair, scant pubic hair and no axillary hair. She was mentally deficient. Her basal metabolism was minus 28 per cent and blood cholesterol was 590 mg. per 100 cc. Roentgen ray examination of the thorax showed a dilated heart with very feeble contractions. The electrocardiogram showed low voltage. The epiphyseal lines of the upper end of the left femur were closed; the epiphyseal cartilage of the upper end of the right femur was still clearly visible. The epiphyseal lines of both iliac crests and ischia were open (Fig. 3).

A remarkable improvement occurred during treatment with desiccated thyroid, which the patient took for the first time (Fig. 2). The thyroid insufficiency apparently began after infancy, since teething, speech and walking followed a normal course.

No. 9147: M. R. was a single woman, 43 years of age (Fig. 5). The sole noteworthy fact in the family history is that her parents were tall. Regarding her personal history, the only thing known is that growth was slow and that she went to school for a year without learning anything. From childhood on, she was always listless and very slow at

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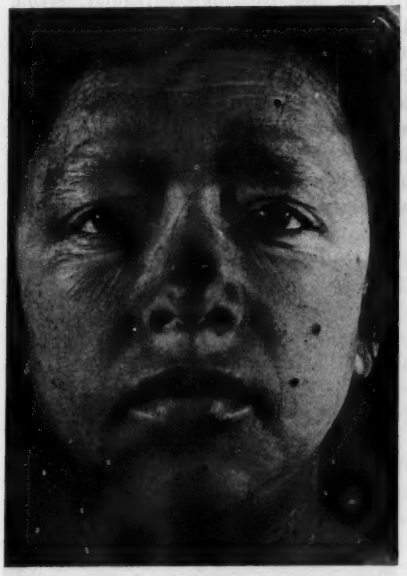

FIG. 1. P.S. before treatment.

FIG. 2. P.S. after treatment with desiccated thyroid.

FIG. 3. Open epiphyseal lines of right iliac crest (P.S. *see* Fig. 1)

FIG. 4. Synostosis of the iliac crest has not yet occurred (M.R. *see* Fig. 5)

her work. The menarche occurred at 13 years of age. Menstruation was regular, lasting from seven to eight days. She had always been constipated, sometimes moving her bowels only once a week. She had always suffered from the cold and perspired very little. Throughout life thirst had been slight and urine output low. She said that her hair was constantly falling out and she was always drowsy. Her appetite had always been poor. She consulted us because of lumbar pains of several months duration.

Physical examination: The patient presented a typical case of myxedema, with a puffy face, moderately enlarged tongue, slowness of speech, dry skin, somewhat scant, coarse, dry scalp hair, scanty pubic hair and no axillary hair. There was a small umbilical

FIG. 5. M.R. before treatment.

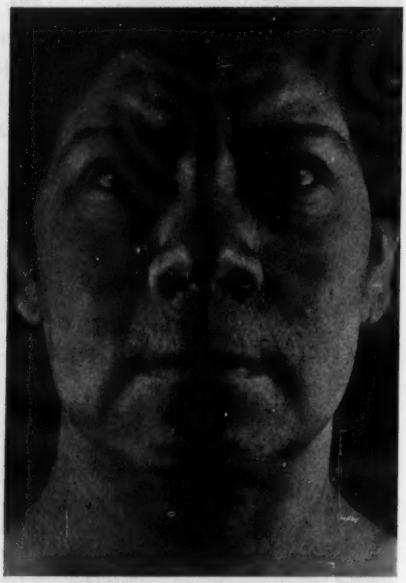

FIG. 6. M.R. after treatment with desiccated thyroid.

hernia. Her height was only 141.0 cm. and her weight was 59.4 Kg. Her mental age was about that of a 7-year-old child. Her basal metabolism was minus 19 per cent and the blood cholesterol was 624 mg. per 100 cc. The electroencephalogram and electrocardiogram showed low voltage. Roentgenographs showed an enlarged heart and unclosed epiphyseal lines of the iliac crest (Fig. 4).

Desiccated thyroid was prescribed in a dose of 0.1 Gm. (1½ grs.) daily. During this treatment the puffiness of the face, the constipation, the lumbar pains, the sensitivity to cold, the dryness of the skin and hair, the drowsiness and listlessness disappeared. The electrocardiogram and electroencephalogram no longer showed low voltage. The blood cholesterol dropped to 198 mg. per 100 cc. A photograph taken six months after starting treatment is shown in Figure 6.

Here we have a case of myxedema which apparently started in childhood and was un-

treated until the age of 42. The early onset is to be deduced from her delayed mental processes, (equivalent to those of a 7-year-old girl) and from her small stature, compared with her tall parents.

No. 3310: This woman was 21 years of age. Her physical and mental development were normal until the age of 8, when growth stopped. At the age of 10 a goiter appeared and was removed by thyroidectomy. After the operation she developed puffiness of the face, listlessness, constipation, lack of perspiration, sensitivity to cold, and impairment of memory and hearing. In spite of lack of treatment with desiccated thyroid, secondary sexual characteristics appeared at the age of 15 or 16 years. The menarche appeared at the age of 19.

Physical examination: Examined for the first time at our Institute at the age of 21 years and 11 months (December 22, 1938) she presented typical myxedematous facies, dry skin, coarse dry hair, absence of axillary hair, and scant pubic hair. Her mental age was that of a girl of 13. Her height was only 132.0 cm. Her basal metabolism was minus 23 per cent. A daily dose of 0.05 Gm. ($\frac{3}{4}$ gr.) of desiccated thyroid was prescribed. After six weeks the puffiness of the face and most of the symptoms of thyroid insufficiency had disappeared. The patient took desiccated thyroid very irregularly, omitting it at times for as long as three years. In spite of the insufficient treatment, menstruation was regular. On May 4, 1945, (nine years after the menarche) vestiges of epiphyseal lines were still visible at the distal ends of the ulna and radius. On July 23, 1947, (eleven years after the menarche) synostosis of the iliac crests was still incomplete.

No. 1338: This woman was 34 years of age. As a child, first and second dentitions and speech were delayed. She began to walk at 4 years of age and showed signs of serious mental deficiency. A colloid goiter was noticed at the age of five. From her early years desiccated thyroid was taken very irregularly. The menarche occurred at 17 years of age. From then on, menstruation continued with some intervals of amenorrhea lasting from two to three months.

Physical examination: Examined at the age of 34, her height was 153.0 cm. Breasts and pubic hair were well developed. A roentgenogram of her pelvis showed the epiphyseal lines of the iliac crests still open.

DISCUSSION

We have presented here the histories of four myxedematous women in whom there was evidently a retarded closure of certain epiphyseal lines, in spite of menstruation having begun a long time previously. The menarche had appeared twelve years before in the first patient, twenty-nine years before in the second, eleven years before in the third, and seventeen years before in the fourth patient.

These facts lead one to suppose that estrogens alone are not sufficient to lead to closure of the epiphyseal lines. It would seem that the thyroid hormone plays a part in this process. It may act directly upon the epiphyseal cartilages in co-ordination with the estrogens. On the other hand, it is also possible that the thyroid hormone, by means of direct or indirect action, may induce the adrenal cortex to secrete some hormone which influences closure of the epiphyses.

In order to explain why the delay in synostosis is greater in panhypo-

pituitarism than in ovarian insufficiency, Albright *et al.* (2) have suggested that the adrenal cortex may contribute to closure of the epiphyses.

The existence of an adrenal cortex deficiency in the course of myxedema is demonstrated by decreased 17-ketosteroid excretion, and by the return of this excretion to normal after the administration of desiccated thyroid. This fact is in accord with the hypothesis we have submitted, namely, that a deficiency of the adrenal cortex may be a factor in myxedema contributing to delay in closure of the epiphyses in spite of normal ovarian function.

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THE URINARY 17-KETOSTEROID LEVELS OF HUMAN LEUKEMIC SUBJECTS[1]

LOUIS LEVIN[2], PH.D.

From the Harry W. Sherman Hematology Research Laboratories, and the Clinical Service of Dr. Raphael Isaacs, Attending Physician in Hematology, Michael Reese Hospital, Chicago

THE dependence of lymphoid organ size and weight upon adrenocortical function has been adequately demonstrated (1 to 6). Recent evidence (7, 8, 9) indicates that functional activity of lymphoid tissue is likewise intimately related to the secretory activity of the adrenal cortex.

Because lymphatic leukemia is characterized in part by hypertrophy and hyperactivity of the lymphoid tissues, it is reasonable to inquire into the possible involvement of the adrenal cortex in the leukemic syndrome. To this end, an evaluation of adrenocortical secretory activity in relation to the leukemic syndrome has been attempted. Two of the available criteria for measuring the activity of the gland have been employed. Adrenal cholesterol concentration, thought (10) to be an indicator of the secretory activity of the cortex, has been determined in mice before and after the induction of lymphatic leukemia. It was found (11) that in mice the disease is associated with a markedly decreased level of adrenal cholesterol, indicating a disturbance in the rate of secretion by the adrenal cortex.

The data presented in the present report were obtained by determination of the urinary 17-ketosteroid excretion levels of leukemic as compared to normal human subjects. The 17-ketosteroids are commonly thought to be largely derived from adrenal cortical hormones by metabolic degradation. The quantities of these substances excreted thus affords a type of measure of adrenal cortical secretory activity.

The leukemic subjects studied were patients hospitalized[3] for various treatments of the leukemic syndrome, most frequently blood transfusion. Insofar as was possible, urine specimens were taken before any "adrenal-activating" treatment (such as irradiation or administration of arsenicals) was instituted. The diagnoses and types of leukemia are indicated in appropriate places. The control subjects were healthy laboratory personnel presumed to be nonleukemic.

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[3] The leukemic subjects were patients of Dr. Raphael Isaacs, who made the diagnoses of the various types of leukemia indicated.

A slight modification of the method of Callow, Callow and Emmens (12) was used for the 17-ketosteroid determinations. It is to be noted that the fractionation procedure included treatment with Girard's Reagent T in order to eliminate nonketonic chromagens.

RESULTS

The data derived from 39 determinations of 17-ketosteroid excretion by 8 leukemic and 6 normal subjects are presented in Table 1.

TABLE 1. URINARY 17-KETOSTEROID EXCRETION BY NORMAL AND LEUKEMIC SUBJECTS

Subject	Age	Diagnosis	Leucocytes		17-Ketosteroid Excretion	
			Total	Blasts	Individual	Average
	(yrs.)		×1000	per cent	mg./24 hrs.	mg./24 hrs.
♂ N.I.	25	normal			6.4; 9.3; 8.9	8.2
♂ H.S.	32	normal			7.6; 4.9; 6.2	6.2
♂ L.L.	37	normal			10.2; 8.4; 7.0; 7.5	8.5
♂ H.A.	67	chronic lymphatic leukemia	70–180	0–1	0.0; 2.5; 2.8; 1.3; 0.8	1.5
♂ P.B.	?	chronic lymphatic leukemia	40–52		2.4	2.4
♂ S.La.	49	acute aleukemic leukemia	0.2–3.9	80–86	2.4	2.4
♂ L.H.	51	acute myelogenous leukemia	5.7–13.8	24–60	10.6; 6.3; 5.0; 6.1; 5.0	6.6
♂ A.F.	28	acute undifferentiated leukemia	21–70	70–95	11.1; 3.9; 9.3; 9.8; 7.6; 7.1; 8.1.	8.1
♀ E.H.	36	normal			4.1	4.1
♀ E.W.	34	normal			6.0; 4.8	5.4
♀ B.S.	30	normal			5.6	5.6
♀ W.	74	chronic lymphatic leukemia			0.9	0.9
♀ F.	50	acute monocytic leukemia	18–120	7–79	2.1; 3.2; 1.7; 3.5	2.6
♀ H.	66	chronic myelogenous leukemia	51–155	2	5.8	5.8

Inspection of the data shows that all three of the lymphatic leukemic subjects excreted markedly reduced amounts of 17-ketosteroids as compared to nonleukemic individuals of the same sex. On the other hand, those patients having leukemia other than the lymphatic variety (one acute myelogenous; one chronic myelogenous; one acute undifferentiated) excreted quantities of 17-ketosteroids of the same magnitude as did normal subjects of the same sex. Two exceptions were the man with acute aleukemic leukemia and the woman with acute monocytic leukemia, both of whom excreted small quantities of 17-ketosteroids. However, it does not seem unlikely that each of these two types may be more closely related, physiologically, to lymphatic than to myelogenous leukemia.

It is to be noted that the 17-ketosteroid excretion levels were not well correlated with clinical conditions. Thus, ♂ A.F., suffering from acute undifferentiated leukemia and almost moribund at the time the urine collections were made, excreted normal amounts of 17-ketosteroids. On the other hand, ♂ H.A., although he had only a mild case of chronic lymphatic

leukemia, excreted greatly reduced quantities of 17-ketosteroids. The lack of correlation between clinical condition and 17-ketosteroid excretion level suggests that debilitation was not the crucial factor here.

The results are not vitiated by the fact that most of the leukemic subjects were in the older age range. It may be noted, for example, that ♂ L H., aged 51, excreted normal quantities of 17-ketosteroids while the urine of ♂ S. La., aged 49, showed a markedly reduced 17-ketosteroid titer. Likewise, ♀ H., aged 66, excreted normal amounts, whereas ♀ F, aged 50, excreted but little 17-ketosteroid.

It thus would appear that only lymphatic leukemia, even in relatively mild chronic cases, is associated with decreased urinary 17-ketosteroid excretion. This finding correlates very well with the report of Dobriner *et al.* (13) that the qualitative distribution pattern of urinary 17-ketosteroids is deranged in lymphatic but not in myelogenous leukemia.

The present results are not interpreted as pointing to the adrenal cortex as a causative agent in lymphatic leukemia. However, when considered together with the results obtained from leukemic mice (11) as well as with the results of other laboratories (13), they suggest that the possible participation of the adrenal cortex in the leukemic syndrome merits further investigation.

SUMMARY

The urinary 17-ketosteroid level of 8 human leukemic subjects has been studied. Markedly subnormal quantities were excreted by 5 of the subjects in whom the diagnoses were chronic lymphatic leukemia (3 cases), acute aleukemic leukemia and acute monocytic leukemia. The 17-ketosteroid excretion was not depressed in three additional patients with leukemia, one with the acute and one with the chronic myelogenous variety and one with the acute undifferentiated type in the terminal stages. These findings indicate a need for further study of the possible participation of the adrenal cortex in the lymphatic leukemia syndrome.

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PROGRAM OF THE 1948 LAURENTIAN HORMONE CONFERENCE

Forest Hills Hotel, Franconia, New Hampshire
September 13-18

Because of limited accommodations, attendance is by invitation. Correspondence should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.

I. STEROID HORMONE METABOLISM "IN VIVO" AND "IN VITRO"

Some aspects of Progesterone Metabolism
DR. G. F. MARRIAN, *University of Edinburgh*
Monday Evening, September 13

Recent Developments in Our Knowledge of Estrogen Metabolism
DR. R. D. H. HEARD and MRS. J. C. SAFFRAN, *McGill University*
Tuesday Morning, September 14

The Metabolism of Androgens by Tissues
DR. LEO T. SAMUELS, *University of Utah*
Tuesday Morning, September 14

The Metabolism of Estrogens with Particular Emphasis on Clinical Aspects of Physiology and Function of Ovarian Hormones
DR. ALBERT SEGALOFF, *Alton Ochsner Medical Foundation*
Tuesday Evening, September 14

II. THE ROLE OF HORMONES IN TISSUE AND BODY METABOLISM

The Antihormone Problem in Endocrine Therapy
DR. JAMES H. LEATHEM, *Rutgers University*
Wednesday Morning, September 15

Integration of the Metabolic Effects of Adrenal Cortical, Thyroid and Growth Hormones
DR. ABRAHAM WHITE, *University of California at Los Angeles*
Wednesday Morning, September 15

The Alterations in Metabolism Incident to Administration of Insulin, Adrenalin and Thyroid Substances, Studied with the Aid of Isotopes

Dr. DeWitt Stetten, Jr., *Harvard University Medical School*

Wednesday Evening, September 15

The Pancreas as the Guardian of the Liver

Dr. Charles H. Best, *University of Toronto*

Thursday Morning September 16

Metabolic Changes in Man Following Adrenal and Pituitary Hormone Therapy

Dr. George W. Thorn, *Harvard University Medical School*

Thursday Morning, September 16

III. NEUROHUMORAL-HYPOTHALAMIC RELATIONSHIPS

Adrenal Function in Mental Disease

Dr. Gregory Pincus, Dr. Hudson Hoagland, Dr. Harry Freeman and Mr. Fred Elmadjian, *Worcester Foundation for Experimental Biology*

Thursday Evening, September 16

Manifestations of Altered Autonomic and Humoral Functions in Psychoneuroses

Dr. Robert A. Cleghorn, and Dr. B. F. Graham, *McGill University*

Friday Morning, September 17

Effects of Hypothalamic Lesions on Water and Energy Metabolism in the Rat

Dr. James A. F. Stevenson, *Yale University*

Friday Morning, September 17

IV. THYROID PHYSIOLOGY AND FUNCTION

Physiologic Reactions of the Thyroid Stimulating Hormone

Dr. Rulon W. Rawson and Dr. William L. Money, *Massachusetts General Hospital*

Friday Evening, September 17

The Metabolism of Iodine as Disclosed by the Use of Radioiodine (I131)

Dr. F. R. Keating, Jr., and Dr. Alexander Albert, *The Mayo Clinic*

Saturday Morning, September 18

Radioiodine as a Diagnostic and Therapeutic Tool in Clinical Medicine

Dr. S. M. Seidlin, *The Montefiore Hospital*

Saturday Morning, September 18

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor; ROY HERTZ. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS

ADRENALS

CLARKE, A. P. W.; CLEGHORN, R. A.; FERGUSON, J. K. W., and FOWLER, J. L. A.: Factors concerned in the circulatory failure of adrenal insufficiency, *J. Clin. Investigation* **26**: 359–363, 1947.

In an effort to determine the factors concerned in the circulatory failure of adrenal insufficiency, changes in blood and interstitial fluid volumes have been observed in 12 experiments upon 8 adrenalectomized dogs in crisis and under adequate treatment with cortical extracts. In conjunction with treatment, attempt has also been made to appraise the influence of desoxycorticosterone acetate and a low salt intake. The plasma volumes were measured by the use of the blue dye T 1824 according to a modification of the procedure of Gregerson and Stewart. Total blood volumes were calculated from the plasma volume. The volume of interstitial cell fluid was determined by the sulfocyanate method. In crisis, plasma volumes were about 30 per cent, total blood volumes 17 per cent and interstitial fluid volumes five per cent to ten per cent below the normal. Three different types of regime for controlling the adrenal insufficiency were used: I. cortin, 1 cc. twice daily plus a diet containing two per cent sodium chloride; II. cortical extract, 4 cc. twice daily, plus one per cent sodium chloride; and III. desoxycorticosterone acetate, 1.25 mg. daily, plus a diet containing one per cent sodium chloride. In each instance, determinations were made during periods of adequate control and in crisis following withdrawal of treatment. Changes in the interstitial fluid varied considerably and were most marked in those animals to which desoxycorticosterone acetate was given. Groups I and II behaved similarly throughout periods of control and crisis. Plasma volume was distinctly higher in group III, and, while values for interstitial fluid varied considerably in all groups and from animal to animal, such changes were most marked in those animals that received desoxycorticosterone acetate. Following the withdrawal of cortin from those animals that had simultaneously received extra salt during the control period, the intake of water and the output of urine were both markedly increased, suggesting interference with the kidney's capacity for excreting a urine containing a high concentration of salt. Observations not reported in the present communication showed "that there is a considerable loss of protein, especially albumin, from the circulating plasma during the development of adrenal insufficiency." On the basis of the data presented, several conclusions have been drawn. Factors concerned in the decreased plasma volume observed in the crisis of adrenal insufficiency include a loss of total

extra-cellular fluid due to a loss of sodium and a retention of potassium and a reduction in plasma proteins with a resulting loss of plasma to the extravascular spaces. The reduction in plasma volume alone does not account for the circulatory failure found in crisis. Cardiac failure, "together with decreased capillary tone" may be responsible "for the severe circulatory failure associated with the apparently moderate decrease in blood volume." Renal excretion accounts for the major portion of the loss of interstitial fluid, but there is also a significant shift to intracellular spaces.—*T. H. McG.*

DOUGHERTY, T. F., and WHITE, A.: An evaluation of alterations produced in lymphoid tissue by pituitary-adrenal cortical secretion, *J. Lab. & Clin. Med.* **32**: 584–605, 1947.

This article represents a review and critical evaluation of the rather extensive work of the authors and of others on the pituitary-adrenocortical regulation of the activity of lymphoid tissue. The known influences of the hormones of these two glands upon lymphoid tissue may be summarized under several headings: 1) *Lymphoid tissue weight:* Injections of adrenotropic hormone of the pituitary caused a significant decrease in the weight of the lymphoid tissue of rats and mice, with the most marked decrease occurring in the thymus gland. The spleen enlarged, due to edema and to the accumulation of degenerating lymphocytes but its Malpighian corpuscles were markedly decreased in size. Adrenalectomy reversed these processes with a marked hyperplasia of all the lymphoid organs. Desoxycorticosterone acetate was without influence on the lymphoid tissues in either the normal or adrenalectomized animal. 2) *Adrenal size and cholesterol content:* A single injection of adrenotropic hormone caused a sharp drop in adrenal cholesterol. Repeated daily injections were associated with an increase in weight and a rise above normal in the values for cholesterol. 3) *Circulating lymphocytes:* Single and repeated insections of adrenotropin produced a lymphopenia, which was maintained throughout the experimental period (observations have been made through 15 days of daily treatment). Adrenalectomized animals with and without desoxycorticosterone acetate therapy showed a significant leucocytosis and lymphocytosis; which was not due to hemoconcentration. The lymphopenic reaction began within one hour and reached its maximum within nine hours following a single injection of the pituitary hormone. In the absence of the adrenal, pituitary adrenotropin exerted no effect upon the circulating lymphocytes. Injections of watery extracts of the adrenal, corticosterone and 11-dehydro-17-hydroxy-corticosterone decreased the lymphocytes of the blood in normal and adrenalectomized animals. Desoxycorticosterone acetate had no such effect. A wide variety of physical and chemical stresses produced lymphopenia in normal rats and mice, but failed to do so in adrenalectomized or hypophysectomized animals. 4) *Circulating polymorphonuclear leucocytes:* No specific influence upon the polymorphonuclear elements of the blood could be observed following the administration of pituitary adrenotropic hormone or steroid hormones of the adrenal cortex. Likewise, hypophysectomy and adrenalectomy were without definitive action. 5) *Circulating erythrocytes:* Both adrenotropin and adrenal cortical hormones caused an increase in hemoglobin and the number of circulating red cells. Conversely, adrenalectomy tended to decrease them. 6) *Histologic changes in lymphoid tissue:* A single injection of adrenotropin or adrenal cortical hormones was followed by dissolution of lymphocytes, reaching its acme within from one to six hours. Concomitantly, there was an increase in the amount and alteration in the structure of the histiocytes. Following the initial dissolution of lymphocytes, cellular debris was removed by macrophages. Within six to nine hours most of the nuclear fragments had been phagocytized, lymphocytic dissolution had ceased, edema had

subsided, and a normal lymphoid structure began to reappear. Within 24 hours there was relatively complete recovery in all lymphoid structures except the thymus. In adrenalectomized animals, neither adrenotropin nor desoxycorticosterone acetate (doses ranging from 0.025 mg. to 2.5 mg.) was capable of eliciting the above responses, but the other adrenal cortical preparations mentioned above were equally active in normal and adrenalectomized subjects. Nonspecific stress stimuli produced changes in the lymphoid tissues of the intact animal, but were without effect in those that had been adrenalectomized. 7) *The release of blood globulin and antibody from lymphocytes:* Adrenotropin and adrenal cortical hormones produced a statistically significant increase in the level of total serum proteins in intact animals. The latter were equally effective in the adrenalectomized animal, but the former failed completely to influence the decrease in the protein of the blood that ordinarily follows adrenalectomy. The increase in protein appeared almost wholly in the beta and gamma globulin fractions thus suggesting the relationship of the phenomenon to factors concerned in antibody formation. When the same hormones were used under equivalent conditions in immunized animals, an increase in the production of antibodies was routinely observed. These alterations in serum protein and in antibody production were always maximal when lymphopenia was highest and lymphocytic dissolution greatest. Extension of these experiments made it clear that the anamnestic response is probably mediated through pituitary stimulation resulting in a release of antibodies from the lymphocytes. The authors conclude that "the role of the adrenal cortex in controlling the rate of release of lymphocyte constituents correlates the functions of the adrenal and of the lymphocyte in resistance. The participation of the adrenal cortex is dependent upon its stimulation by pituitary adrenotropic hormone."—*T. H. McG.*

MAYCOCK, R. L., and ROSE E.: Insensitivity to epinephrine in a patient with a functioning tumor of the adrenal medulla, *Am. J. M. Sc.* **213**: 324, 1947.

The case history is reported of a 42-year-old male who experienced one to 16 attacks daily for two and one-half years before admission for paroxysmal hypertension and associated symptoms. The physical examination was essentially normal, and the blood pressure was 130/80 mm. of mercury when the patient was free from attacks. Attacks were often brought on by physical exercise or changes in position. Detailed clinical description of the attacks is given. A tumor weighing 42.8 Gm. and containing 76.5 mg. of free epinephrine was successfully removed from the region of the right adrenal. Histologically the morphology was consistent with the usual pheochromocytoma or adrenal paraganglioma. Prior to operation, studies were made to discover what stimuli were effective in producing an attack. Typical paroxysms were induced by exercise and postural changes, such as having the patient bend and twist his waist or press his knees into his abdomen, and by the subcutaneous injection of 10 mg. of acetyl-beta-methyl choline. Emotion, manual massage of the adrenal area, injections of sterile water, and inhalation of a perle of amyl nitrite were ineffectual in producing an attack. The most striking demonstration was the insensitivity of the patient to epinephrine: 2.5 cc. of a potent 1:1000 solution were needed to produce a blood pressure rise to 220/114 and the symptoms that usually occurred with a mild attack. After the tumor had been removed, injection of 0.25 cc. of 1:1000 epinephrine solution subcutaneously induced a rise in blood pressure to 172/98 with all of the previous symptoms of a typical attack. This time, however, he complained of nervousness which he had not experienced before even during his worst episodes. Twenty-three minutes after the onset of a severe attack, the

serum potassium level was depressed to 2.9 m. Eq./100 cc. (normal range 3.8 to 4.3); when no attack was present, the level was elevated to 5.0 m. Eq./100 cc.; at operation the potassium content of the rectus muscle was found to be 172.0 m. Eq./Kg. of wet muscle (normal 82 m. Eq./Kg.). During an attack 0.45 mg. of conjugated epinephrine was excreted per 100 cc. of urine. The amount of choline esterase in the blood was normal during the interval between attacks. Additional data are given, including the values for other chemical constituents of the blood, and the electrocardiograms and ballistocardiograms before, during, and after attacks. The authors conclude that the patient was hyposensitive to epinephrine action while the medullary tumor was present and was normally sensitive to this drug when the tumor had been extirpated. They suggested that the demonstration of such insensitivity may serve as a useful and harmless test for the diagnosis of such tumors.—*E.C.R., Jr.*

SELYE, FRANCES L.: Biochemical changes in hypertension, *Canad. M.A.J.* **57**: 325, 1947.

In this study an attempt is made to find criteria for an adrenocortical etiology in certain cases of "essential" hypertension. On the basis of animal experiments of H. Selye, and of clinical studies in Cushing's syndrome (Kepler), a high Na/Cl ratio in the serum is considered suggestive of hypersecretion of "mineralo-corticoids." Several of these patients also had high "glycocorticoid" with low 17-ketosteroid excretion. Ammonium chloride therapy was given under adequately controlled conditions, including control periods of placebo medication. This therapy was suggested by the fact that in animal experiments vascular changes from desoxycorticosterone plus salt administration failed to occur when ammonium chloride was given simultaneously. The results of this therapy were encouraging.—*K.E.P.*

GONADS

GUTERMAN, H. S.: Influence of arginine on oligospermia, *Proc. Soc. Exp. Biol. & Med.* **65**: 176–178, 1947.

Observations were made on 23 patients with oligospermia. Eighteen received from 1.8 Gm. to 2.7 Gm. of arginine and minimal amounts of lysine, pyridoxine and tryptophane in tablet form daily for eight months and five patients received no specific therapy. All of the men had histories of adequate protein intake. No improvement in semen quality which could be attributed to treatment was produced and it was concluded that amino acid therapy for oligospermia should be reserved for patients who exhibit or give the history of inadequate protein intake.—*F.N.A.*

HARTMAN, C. G., and CORNER, G. W.: Removal of the corpus luteum and of the ovaries of the Rhesus monkey during pregnancy: observations and cautions, *Anat. Rec.* **98**: 539, 1947.

In a series of experiments on the physiology of reproduction in the monkey it was found that the corpus luteum is not essential to the continuation of pregnancy after the twenty-fifth day. Complete surgical removal of the ovaries in the monkey is attended with great difficulty and the authors hold that in primates, total removal must be proven by the subsequent absence of cyclic phenomena, by microscopic evidence that the excised ovaries were removed intact and by a thorough search for residual ovarian tissue.—*B.L.B.*

KIMMEL, G. C. and WING, R.: Sexual precocity and accelerated growth in a child with a follicular cyst of the ovary, *J. Ped.* **30**: 686, 1947.

The authors report the case history of a 25-month-old girl who showed all the changes observed in granulosa-cell tumor of the ovary, including a greatly increased urinary estrogen level. At operation, the right ovary was found to consist chiefly of a cyst 4 by 3 cm. in diameter which had destroyed most of the ovarian tissue. Careful examination of the cyst revealed no granulosa-cell tumor and it was classified as a follicular cyst of the ovary. The pathologist suggested that sufficient estrogen to cause the changes noted in this patient might have been produced by the granulosa cells which lined the cyst. During the following four years there has been no recurrence of vaginal bleeding; the breasts, the nipples and the labia have regressed to their normal size; and the estrogen content of the urine has returned to and remained at a normal level. The patient has remained above average height and weight during these subsequent years. No abnormal values for urinary ketosteroid excretion were found at any time.—*E.C.R., Jr.*

LEONARD, S. L.; PEARLMAN, P. L., and KURZROCK, R.: Relation between time of fertilization and follicle cell dispersal in rat ova, *Proc. Soc. Exp. Biol. & Med.* **66**: 517–518, 1947.

Following the discovery that the enzyme hyaluronidase from sperm disperses the follicle cells of recently ovulated mammalian ova, a concept has arisen that the action of hyaluronidase facilitates fertilization by denuding the ova prior to or simultaneously with sperm entry. The observations reported in this paper indicate no mass removal of the follicle cells prior to fertilization and that sperm penetration precedes the gross denudation of the ovum in the rat. Female rats were bred and the ova examined microscopically 12 to 26 hours later. In another experiment 0.2 cc. of hyaluronidase from bull or rat testes, in concentrations of 30 to 60 turbidity reducing units per cc., was introduced into each horn of the uterus of a rat in heat and the horns ligated near the cervix to prevent leakage. It was found that fertilization of the rat ovum occurred before mass displacement of the surrounding follicle cells; denudation of the ova occurred subsequently. Hyaluronidase introduced into the uterus did not pass into the tubes and denude the ova and it appeared that only the enzyme associated with the sperm which reach the oviduct, disperses the follicle cells.—*F.N.A.*

REIFENSTEIN, E. C., JR., and ALBRIGHT, F.: The metabolic effects of steroid hormones in osteoporosis, *J. Clin. Investigation* **26**: 24–56, 1947.

The authors define osteoporosis as "that form of undermineralization of bone in which the primary defect is a hypofunction of the osteoblasts in laying down bone matrix." Sharply distinguished are osteomalacia, where there is a failure in mineralization; and hyperparathyroidism, where there is an increased destruction of bone. Osteoporosis in the sense used occurs in malnutrition (protein), Selye's adaptation syndrome, idiopathic osteoporosis, acromegaly, Cushing's syndrome, disuse atrophy, old age, and, commonest of all, the postmenopausal state. Twelve patients with osteoporosis are described in detail. In eleven the results of therapy with estrogens, androgens and progesterone are described. These subjects represent the last four categories mentioned above. Estrogens caused a decrease in calcium and phosphorus excretion in the subjects of all four groups. They usually caused a decreased fecal calcium and phosphorus as well. A moderate but not well sustained decrease in urinary nitrogen and 17-ketosteroids

was observed. Effective dosages for estradiol benzoate intramuscularly ranged from 1.66 mg. every three days to 3.32 mg. daily; and, for stilboestrol orally, from 1 mg. to 15 mg. daily. The smallest of these doses was usually effective with very little if any increase in activity as increments in dosage were made. No qualitative difference could be observed between the natural and the synthetic estrogen. The effects of the hormones on calcium and phosphorus balance were demonstrable by the sixth day, reached their maximum around the thirtieth day, and persisted from one to two months following the cessation of treatment. Androgens in the form of testosterone propionate intramuscularly (25 mg. to 50 mg. daily) and methyl testosterone by mouth (40 mg. to 100 mg. daily) decreased the urinary and fecal excretion of calcium and phosphorus in osteoporosis. These effects made their appearance about the fifth or sixth day as with the estrogens, but their acme occurred somewhat later, and they disappeared more slowly after withdrawing the drug. The decrease in urinary nitrogen was marked and prolonged. Testosterone propionate and methyl testosterone were equally effective. When androgen and estrogen were combined in the postmenopausal and senile groups the effect was greater than that observed following the administration of either alone. Progesterone, intramuscularly, in doses of 10 mg., 25 mg., and 100 mg. daily, for as long as 36 days, was without appreciable effect upon the urinary and fecal excretion of calcium, phosphorus or nitrogen, whether it was used alone or in combination with one of the other hormones. Except in Cushing's syndrome, the beneficial effects of estrogens upon the bony disturbance were more marked than those of testosterone. The authors call particular attention to their regime for the management of the postmenopausal type of osteoporosis. In order to avoid troublesome bleeding and to decrease the risk of inciting a precancerous lesion into full activity, treatment is usually interrupted every four to seven weeks for one to two weeks, and often a short course (five days) of progesterone is employed during this interval. Vaginal smears are used periodically in an effort to detect any neoplastic change early.—*T.H. McG.*

TAYLOR, R. D.; CORCORAN, A. C., and PAGE, I. H.: Menopausal hypertension: A critical study, *Am. J. M. Sc.* **213**: 475, 1947.

Since the concept "menopausal hypertension" appeared to have been derived merely from accumulated impressions rather than from systematic study, the authors studied the incidence of hypertension in 200 menopausal women, 179 of whom had been surgically castrated and all of whom desired relief of menopausal symptoms. The results showed that arterial hypertension is no more common in menopausal women than in the general population. The authors believe that "vasomotor instability," as exhibited by "hot flashes," perspiration, and tachycardia, is not necessarily associated with hypertension and its alleviation by estrogens need not affect arterial pressure. In general, the menopause seemed to intensify preexisting psychoneuroses. Despite severe neurotic behavior, hypertension did not develop within three or more years except in 6 cases. The authors conclude that the relationship between the menopause and hypertension is incidental, and that loss of ovarian secretion is neither a primary nor a contributory cause of arterial hypertension.—*E.C.R., Jr.*